An Observational Study to Determine the Incidence of Thyroid Dysfunction Among Pregnant Women in Northern Tasmania: Effect of Thyroid Status on Maternal, Foetal and Neonatal Outcomes

Abdul Majeed, MBBS

Submitted in fulfilment of the requirements for the degree of Master of Biomedical Science

School of Health Sciences
Faculty of Health

University of Tasmania

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Full Name: Abdul Majeed

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List of abbreviations

AACE	American Association of Clinical Endocrinologists
AI	Adequate intake
APH	Ante-partum haemorrhage
ATA	American thyroid association
BD	Becton Dickenson
C5	Fifth cervical vertebra
CB-TSH	Cord blood TSH
EDTA	Ethylenediaminetetraacetic acid
ES	Endocrine society
FBC	Full blood count
Fe^{2+}	Heme iron
Fe^{3+}	Non-heme iron
FT3	Free triiodthronine
FT4	Free thyroxin
GDM	Gestational diabetes mellitus
GHTN	Gestational hypertension
HCG	Human chorionic gonadotropin
HTN	Hypertension
ICCIDD	International council for the control of iodine deficiency disorders
ICP-MS	Inductively coupled plasma mass spectroscopy
IDA	Iron deficiency anaemia
IDD	Iodine deficiency disorder
IH	Isolated hypothyroxinemia
IOM	Institute of medicine

IPH	Intrapartum haemorrhage
IQ	Intelligence quotient
IRR	Incidence rate ratio
IUFGR	Intra-uterine foetal growth restriction
LC/MS/MS	Liquid chromatography/tandem mass spectrometry
LGH	Launceston general hospital
LSCS	Lower segment caesareans section
MCV	Mean corpuscular volume
NHMRC	National Health and Medical Research Council
NIS	Sodium/iodide symporter
OH	Overt hypothyroidism
PPH	Postpartum haemorrhage
RDA	Recommended dietary allowance
RDS	Respiratory distress syndrome
RNI	Recommended nutrient intake
ROC	Receiver operating curve
RPA	Royal Prince Alfred
SCH	Subclinical hypothyroidism
T1	First thoracic
T3	Triiodthyronin
T4	Thyroxin
TBG	Thyroxin binding globulin
TG	Thyroid globulin
TRH	Thyrotropine releasing hormone
TISP	Tasmanian iodine supplementation program

TPL	Threatened premature labour
TPO	Thyroid peroxidase
TSH	Thyroid stimulating hormone
UI	Urinary iodine
UIC	Urinary iodine concentration
UIE	Urinary iodine excretion
USA	United States of America
USI	Universal salt iodisation
WHO	World Health Organisation

Summary of the thesis

This thesis comprises four chapters; Literature Review, Materials and Methods, Results and Discussion/Conclusions. It represents the work conducted by the candidate during the period April 2013 and May 2015.

Chapter one is a critical review of the scientific literature related to iodine and iodine deficiency. Firstly, it highlights that Tasmania has had a history of thyroid disease in the community, including iodine deficiency. More recently, a Tasmanian study reported that children born to iodine-deficient mothers suffered impaired cognitive development.

Secondly, it examines the changes occur in thyroid function during pregnancy and whether these have any influence on pregnancy outcomes. It further analyses different opinions regarding the role of iron in pregnancy as well as the effect of iron deficiency anaemia on pregnancy. It also explores the feasibility of iodine level surveillance in childbearing women as well as potential applications of cord blood in foetal thyroid assessment.

Studies of thyroid function and iron deficiency during pregnancy in Tasmania are limited. Furthermore, the application of general screening for thyroid function/dysfunction during and prior to pregnancy remains controversial. Thus, the overall aim of the present study was to determine the incidence of thyroid dysfunction during pregnancy and to investigate whether any possible relationships exist between low thyroid status and complications occurred during pregnancy and labour.

Chapter two summarises the study design, subject recruitment, materials, and methods used during this study. It further describes sample processing, tests used during screening, including full blood count, iron studies, maternal, foetal thyroid function tests (TSH, FT4, FT3 and anti-thyroid peroxidase antibodies), and urinary iodine concentration.

The results chapter primarily presents the data of the study in a series of tables and figures, including description of the study population and study outcomes. A response rate of 28.9% was demonstrated, with 609/636 (95.7%) of participants successfully screened for thyroid status. Next, incidence of thyroid abnormalities in the study population was demonstrated; 22 (9 with TSH >3 mU/L, 13 with TSH 2.5-3mU/L) and 75 with FT4<8.93.4%, representing 3.4% of low thyroid status and 12.3% of isolated hypothyroxinemia, respectively.

Furthermore, possible association of maternal and foetal complications with the thyroid status was also shown; prolonged labour (IRR 3.31; 95% CI 1.13 to 9.69; P<0.05) and gestational diabetes (IRR 1.69; 95% CI 0.55-5.25; P=0.36). It also demonstrates that the study population is iodine deficient; median UIC=117µg/L with 11.2% of them having developed isolated hypothyroxinemia (FT4<8.9 mU/L) during pregnancy.

Additionally, CB-TSH results of 402/615 (65%) of babies born to this study cohort was analysed. It shows a significant decrease of TSH level in the cord blood of babies born to participants with bleeding complications (APH/IPH) (Mean (SD)

4.91(2.16); 95% CI -5.48 to -2.48; P<0.001) compared to CB-TSH of babies born to participants without bleeding (Mean (SD) 9.40(5.97)). Although this may not reflect abnormal foetal thyroid function, it does demonstrate a strong thyroid reaction to maternal ante-/intra-partum bleeding that needs further investigations.

The last chapter discusses the main outcomes of the study and shows how the incidence of low thyroid function of this study is unexpectedly low but in line with international figures. It also discusses the importance of iodine surveillance as well as general screening for thyroid function prior or at early pregnancy. The chapter concludes by emphasising the importance of the incidence of thyroid disorder in this population in Northern Tasmania, and the possibility of an increased incidence of antenatal complication in association with low thyroid status being equivocal. Since the main limitation of this study was the small sample size, more statistically significant outcomes are anticipated with larger scale studies in the future.

1 LITERATURE REVIEW

1.1 Introduction

Management of a swollen thyroid gland (Goitre) was first explained by the ancient Chinese 3500 years ago (Temple, 1999). Iodine deficiency was later recognised to be the main cause of goitre in some areas of mountainous terrain and other inland places (Vanderpas, 2006, Richards and Stewart, 2007, Zimmermann, 2010). Tasmania has a strong history of battling with iodine deficiency and goitre (Gibson, 2006). The magnitude of the disease was clearly evidenced by pictures and reports in a Tasmanian publication (Richards and Stewart, 2007). Furthermore, early reports of medical and surgical treatment for goitre in the Launceston General Hospital (LGH), were made in late 19th century (Richards and Stewart, 2007). It has been advised that any health condition caused by iodine deficiency which is preventable by iodine supplementation is now classified as an Iodine Deficiency Disorder (IDD), by the World Health Organisation (WHO) (Andersson et al., 2005, Andersson et al., 2012).

The prevalence of thyroid diseases in a form of subclinical hypothyroidism during pregnancy is estimated to be 1.5-4% (Allan et al., 2000, Casey et al., 2005, Negro and Mestman, 2011). While the physiology and pathology of the thyroid gland has been well studied, results of thyroid function tests in laboratories varied considerably during pregnancy (Burrow, 1990, Glinoer, 1999, Casey and Leveno, 2006, Gärtner, 2009, Hall, 2010). Pregnancy was described as a state of significant hormonal changes that could result in elevated thyroid stimulating hormone (TSH) and suppressed thyroxin (T4) (Glinoer, 1999). Thus, maintaining balance between these changes in iodine replete areas was found to be the key factor for normal progress of pregnancy and resulted in a better outcome (de Benoist et al., 2003, Glinoer, 2004).

It was also reported that maternal thyroid disease during pregnancy has a negative impact on foetal growth and offspring's neuropsychological development (Cranefield, 1962, Koibuchi and Chin, 2000, Delange, 2001, Patel et al., 2011). Possible association between thyroid disease and iron deficiency anaemia has been speculated (Hess et al., 2002, Zimmermann and Köhrle, 2002). A recent study conducted at the LGH found that 20% of pregnant women in Northern-Tasmania had iron deficiency anaemia (IDA) (Khalafallah et al., 2010). Since pregnancy increases the demand for many nutrients including iodine and iron, a possible link between iron deficiency anaemia and IDD during pregnancy was supported (Kramer, 2003).

It is agreed that goitre and hypothyroidism are most common in iodine deficient areas (Glinoer, 1997, Lazarus and Okosieme, 2000, Glinoer, 2004, Gibson, 2006, Vanderpump, 2010). However, clinicians only consider testing for thyroid dysfunction in pregnancy if one or more of the following situations occur; symptoms exist that suggest the condition; there is a family history of the disease; or there is the presence of an associated medical condition (Abalovich et al., 2002, Reid et al., 2010). Nearly a third of women were overlooked when applying a case-finding approach rather than routine screening (Vaidya et al., 2007). Despite this, general screening for thyroid function prior or during pregnancy is not a standard practice in Australia generally and Tasmania in particular. Hence, the actual incidence of thyroid diseases and its possible complications within the pregnant population of Tasmania is not yet known. It is also worth mentioning that the study is specific for a geographical region (Tasmania) that has a persistent history of iodine deficiency(Hynes et al., 2004). Other studies conducted in different regions of

Australian were either among iodine sufficient population or among unknown iodine status (Gilbert et al., 2008b, Schneuer et al., 2012, Ong et al., 2014).

1.2 Iodine and Iodine Deficiency Disorder (IDD)

1.2.1 Background

Iodine was discovered in the early 19th century by Bernard Courtois, a French chemist (Richards, 1995). Davy, a British chemist and Gay-Lussac of France however named the element "Iodine/Iod" in reference to a purple colour in Greek language (Kelly, 1961, Richards, 1995). Hypothyroidism as a result of goitre is believed to occur predominantly in regions of mountainous terrain where past glaciations and heavy rainfall has resulted in the leaching of soil iodine (Goldschmidt, 1952, Andersson et al., 2005, Zimmermann, 2010). Geographical isolation and largely self-sufficient food production in a moderately to severe iodine deficient region worsens the problem (Assey et al., 2006). Tasmania falls under such a category and is known to be a typical endemic goitre area (Gibson, 2006, Richards and Stewart, 2007).

Apart from natural causes of iodine deficiency, replacing iodine-based disinfectants by contemporary non-iodine ones was suggested to be another reason for low-iodine milk production in Tasmania (Richards and Stewart, 2007). The author also proposed that introduction of fast food in late 20th century probably had a significant role in re-appearance of IDD in both developed and developing countries (Richards, 1995, Richards and Stewart, 2007). IDD has been recognised as a public health problem affecting many communities globally (Andersson et al., 2012). Australia, including

Tasmania, was found to be mildly iodine deficient according to a nationwide study (Li et al., 2006).

Internationally, iodine supplementation programs were first implemented in the 1920s by the United States of America (USA) and Switzerland (Marine and Kimball, 1920). However, this was not widely adopted until the World Health Organisation (WHO) launched a Universal Salt Iodisation (USI) programme in the early 1990s (Andersson et al., 2005). In Tasmania, it was only between the 1950s and 1960s when iodine tablets were dispersed to people in order to contain goitre and prevent IDD (Gibson, 2006).

While fortifying bread with iodised salt was recommended by the Tasmanian Iodine Supplementation Program (TISP) launched in 2001, it was then legislated to be a mandatory fortification in 2006 (Richards, 1995, Burgess et al., 2007, Richards and Stewart, 2007). However, it is worth noting that both studies were conducted during voluntary fortification period. Nevertheless, mild iodine deficiency in Tasmania was found to be determined, concluded by Menzies Research Institute, Hobart, Tasmania (Hynes et al., 2004).

In addition, a nine year follow up study of school-aged children who were born to iodine deficient mothers revealed some cognitive disorder, i.e., reading difficulties when compared to peers of similar age and school level (Hynes et al., 2013). This indicates that earlier detection and prevention of iodine deficiency disorders in future mothers can reduce risk of cognitive disorder in their children.

1.2.2 Iodine & iodine metabolism

Recognition of iodine's importance was related to its essential role in the production of thyroid hormones; thyroxin (T4) and triiodthyronin (T3). In the stomach and duodenum, >90% of iodine in the form of iodide was found to be rapidly absorbed (Taurog, 2000, Trumbo et al., 2001). While the half life of iodine was reported to be 10 hours in normal health and adequate nutritional conditions, it was found to be reduced in case of iodine deficiency (Taurog, 2000).

Iodine uptake in the thyroid gland is dependent on its supply, with 10% of iodine uptake enough in case of iodine sufficient supply and >80% of uptake could be required in insufficient iodine supply (DeGroot et al., 1975). The sodium/iodide symporter (NIS) system was found to be responsible for iodide transfer into the thyroid (Eskandari et al., 1997).

In the colloid, the enzyme thyroid peroxidase was established to play a crucial role in iodine coupling and the production of thyroid globulin (TG), T3 and T4 (Eskandari et al., 1997, Taurog, 2000, Hall, 2010). Circulating T3 and T4, after reaching the target organs they release iodine which enter the plasma iodine pool where it is taken up again by the thyroid or excreted (90%) by the kidneys (Taurog, 2000), (Figure 1-1).

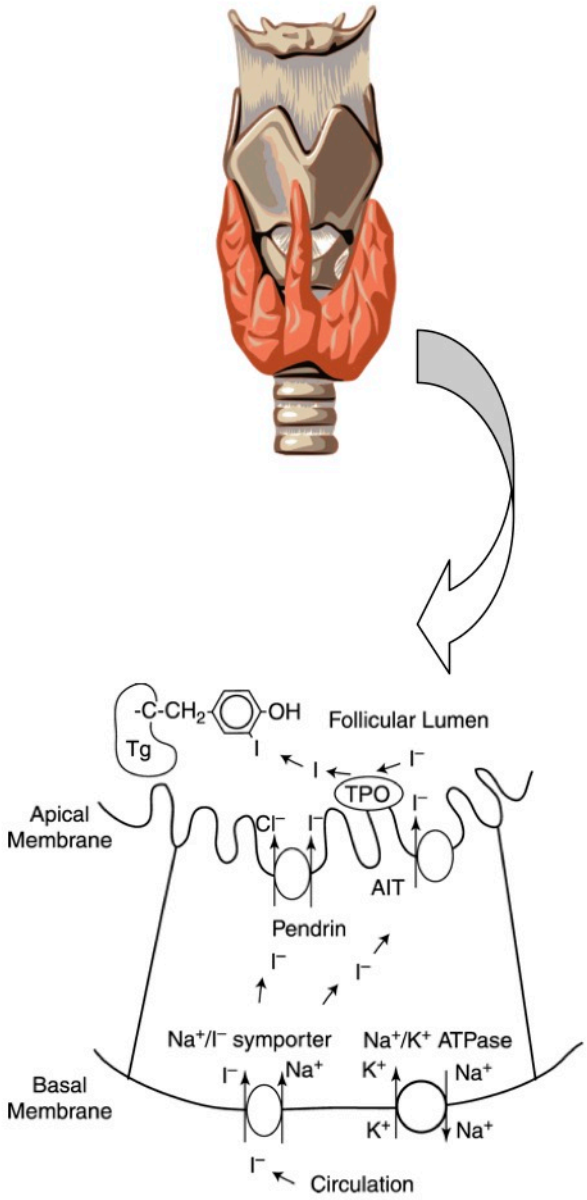

Figure 1-1 Iodine metabolism and transport

At the basal membrane NIS regulate transfer of iodine from the circulation into the thyroid cell. The apical membrane is where the thyroid peroxidase enzyme helps the iodine to couple up with the thyroid globulin.

1.2.2.1 Iodine sources

Iodine is commonly available in seawater, seaweeds and seafood. It mainly exists in the sea water in oxidised form, and in a lesser percentage it reaches the soil which is again drained back to the sea by the effect of rain (Goldschmidt, 1952). Thus, crops grown in these washed-off soils would be low in iodine concentration. Consequently, human and animals consuming food grown in these iodine-poor soils are likely to develop IDD (WHO, 2001). A study comparing iodine content in crops grown in iodine-deficient soil and crops grown in iodine-sufficient soil, resulted in 10 μg/kg and 1 mg/kg of dry weight of iodine, respectively (Assey et al., 2006). Iodine-deficient soils are common in inland regions, mountainous areas, and places with frequent flooding, but can also occur in coastal regions (Vitti et al., 2003, Andersson et al., 2012).

Since consuming large amounts of seaweed was a normal diet for people living on the coastal regions of Japan, remarkably high iodine intakes amounting to 50 to 80 mg/day have been reported in this population (Zimmermann, 2009). In the mid-1990s in the United States, the median intake of iodine from food was estimated to be 240 to 300μg/day for men and 190 to 210μg/day for women. Since salt iodisation programs have been applied by most countries including Australia, bread has been the major source for iodine. In addition to bread, milk was mentioned as well to be a good source for iodine in the USA and Europe (Pearce et al., 2004, Haldimann et al., 2005). Other publications reported that, using iodophors milk in cleaning milk cans and cows teats increased the native iodine content of dairy products (Phillips, 1997, Richards and Statham, 2007).

1.2.2.2 Iodine requirements

Definitions related to nutritional requirements were outlined by the Institute of Medicine (IOM) of the USA (Trumbo et al., 2001). The estimated average requirement (EAR) is the daily iodine intake that meets the requirement of half of the healthy individuals in a particular life stage (Trumbo et al., 2001). The recommended dietary allowance (RDA) however, was defined to be the average daily intake sufficient to meet the iodine requirement of 97–98% of healthy individuals in a life stage (Trumbo et al., 2001). The adequate intake (AI) was used if there was insufficient scientific evidence to calculate an EAR (Trumbo et al., 2001).

Whilst these definitions were widely accepted in the US, the recommended nutrient intake (RNI) was adopted by WHO (Zimmermann, 2009). RNI was defined as the intake estimated to cover the needs of "nearly all" healthy individuals in the specified life stage (Trumbo et al., 2001). It has been recommended for men, non-pregnant and non-lactating women as the adequate daily iodine intake was 150 μg/d (Zimmermann, 2009).

During pregnancy, increased production of maternal T4, retained iodine in foetal thyroid and increased renal clearance, were thought to be the main reasons for increased iodine demand (Glinoer, 1997). The latest and ongoing WHO's recommendation for daily iodine supplementation during pregnancy and lactating has been reported to be 250 μg/day (Andersson et al., 2007). While iodine supplementation for pregnant and breastfeeding women in Australia and the New Zealand in 2006 was 220 μg/d and 270 μg/d respectively, the National Health and Medical Research Council (NHMRC) instead recommends that 150 μg/day of iodine

supplementation was safe and effective for pregnant and breastfeeding women (NHMRC, 2010). This relatively lower than WHO's recommendation was further explained by the NHMR; avoiding any possible foetal thyroid depression.

1.2.3 IDD

This phenomenon was identified globally and defined by the World Health Organisation (WHO) as any illness related to a thyroid disorder that could be prevented by dietary iodine supplementation (Zimmermann et al., 2000, Andersson et al., 2007). While severe iodine deficiency in pregnancy can result in irreversible brain damage to the foetus (cretinism), mild deficiency can lead to a decreased intelligence quotient (IQ) in school aged children (Chan and Kilby, 2000, Koibuchi and Chin, 2000, Delange, 2001, Burgess et al., 2007).

The magnitude of IDD is at a global scale, with approximately 1.5 billion people affected (Hetzel, 1983, de Benoist et al., 2003, Andersson et al., 2005, Andersson et al., 2012). Although iodine deficiency was found to have a high prevalence in developing countries, about 600 million people in Europe were reported to suffer from a mild-to-severe level of iodine deficiency (Vitti et al., 2003). Subsequently, a safe, cost-effective and sustainable strategy was conducted using salt iodisation method to ensure adequate iodine intake in all affected areas (Andersson et al., 2005, Andersson et al., 2012).

The iodised salt program was adopted by most countries around the globe, with an estimated 71% of the world's population benefiting from this strategy (Andersson et al., 2012). Using Urinary Iodine Excretion (UIE) levels, recommendations were

made by WHO/International Council for the Control of Iodine Deficiency Disorders (ICCIDD): iodine replete, UIE ≥ 100 μg/L; mild iodine deficiency, UIE 50–99 μg/L; moderate iodine deficiency, UIE 20–49 μg/L; severe iodine deficiency, UIE < 20 μg/L) (Andersson et al., 2012). Although 24h UI is a better indicator, it is not practical to be used when conducting large scale studies (World Health Organization, 2007).

During a period of voluntary iodine fortification in Australia, a cross-sectional study involving 1709 school-aged children from 88 schools comprising almost equal percentages of boys and girls, reported a borderline iodine deficiency with a median UIE of 104 μg/L (Li et al., 2006). It was reported that children in South Australia, NSW and Victorian were either border line or mildly iodine deficient, with median UIE levels of 101 μg/L, 89 μg/L and 73.5 μg/L, respectively (Li et al., 2006). Queensland and Western Australian children were found iodine sufficient with median UIE levels at 136.5 μg/L and 142.5 μg/L, respectively.

The median urinary iodine concentration (UIC) in 285 pregnant women who were enrolled prior to salt fortification with iodine and 517 enrolled after iodised salt use in most of Tasmania's bakeries showed 76 μg/L and 83,5 μg/L, respectively, indicating a mild iodine deficiency according to the WHO classification (NHMRC, 2010, Burgess et al., 2007). A follow-up study was performed that evaluated the mental development of school-aged children who were born to iodine deficient mothers (UIC <150 μg/L) in the previous study (Hynes et al., 2013). The study resulted in reductions of 10.0%, 7.6% and 5.7% in spelling, grammar and in English-literacy, respectively, when compared with peers who were born to iodine sufficient

mothers (Hynes et al., 2004, Hynes et al., 2013). It was therefore suggested that even mild iodine deficiency during pregnancy could have long-term adverse affects on foetal cognitive functions, that had not been sufficiently were not compensated by the iodine sufficiency strategy that commenced in the state in 2001 (Hynes et al., 2013).

1.3 Thyroid Gland

The thyroid is one of the largest glands in the human body, regulates metabolism and growth (Hall, 2010). It produces hormones; thyroxin, triiodthyronin and calcitonin. Circulating in a very small amounts in the body, they have specific effects on other organs and systems e.g. cardiovascular, neuropsychology, musculoskeletal and sexual (Hall, 2010).

1.3.1 General anatomy and physiology

It is located on the front of the neck, hugging the thyroid cartilage and the trachea from the level of fifth cervical vertebra (C5) to the first thoracic (T1), thus occupying the front surface of the second to fourth tracheal rings. Butterfly, H and U are the most common shapes of this gland, formed by two elongated side lobes and a small lingual (isthmus) lobe that connects the lateral lobes together. The average weight of the thyroid gland is 25-30g in adults, it is however may enlarge during pregnancy and menstruation. Its blood supply comes from the superior and inferior thyroid arteries and innervated by parasympathetic and sympathetic fibres from vagus nerves and the sympathetic trunk. Biosynthesis of the thyroid hormones take place in the thyroid functional unit which consists of a monolayer of epithelial cells that is filled

with thyro-globuline (colloid) (Marine and Kimball, 1920, Kopp, 2005, Braverman and Cooper, 2012). For the production of thyroid hormones, Iodine in the form of iodide (I^-) is required, hence transported into this unit using NIS (Eskandari et al., 1997, Hall, 2010). The thyroid, is responsible for production of a great number of protein enzymes, structural protein, transport protein and other substances, which increase general activity throughout the body (Bizhanova and Kopp, 2009).

They stimulate Carbohydrates and fat metabolism as well as increases utilisation of vitamins (Hall, 2010). Thyroid hormones metabolic effect was demonstrated in an animal experiments, number and size of mitochondria increased in response to thyroid hormones ingestion, increases the rate of basic body metabolism up to 60-100 percent (De La Vieja et al., 2000, Bizhanova and Kopp, 2009, Hall, 2010). The thyroid hormones, are also responsible for brain maturation and neuropsychological development (Chan and Kilby, 2000, Glinoer, 2004, de Escobar et al., 2004, Hall, 2010, Yassa et al., 2010). Maintaining thyroid hormones and its functions within normal and regular secretory level, gained by a specific negative feed-back mechanism within the hypothalamus-pituitary-thyroid axis (Glinoer, 2004, Hall, 2010).

Secretion of thyroid hormones T4 and T3 is directly stimulated by the TSH; TSH is secreted from the anterior lobe of the pituitary gland and is stimulated by the Thyrotropine releasing hormone (THR) in the hypothalamus, hence is called hypothalamic-pituitary-thyroid axis (Chiamolera and Wondisford, 2009), (Figure 1-2).

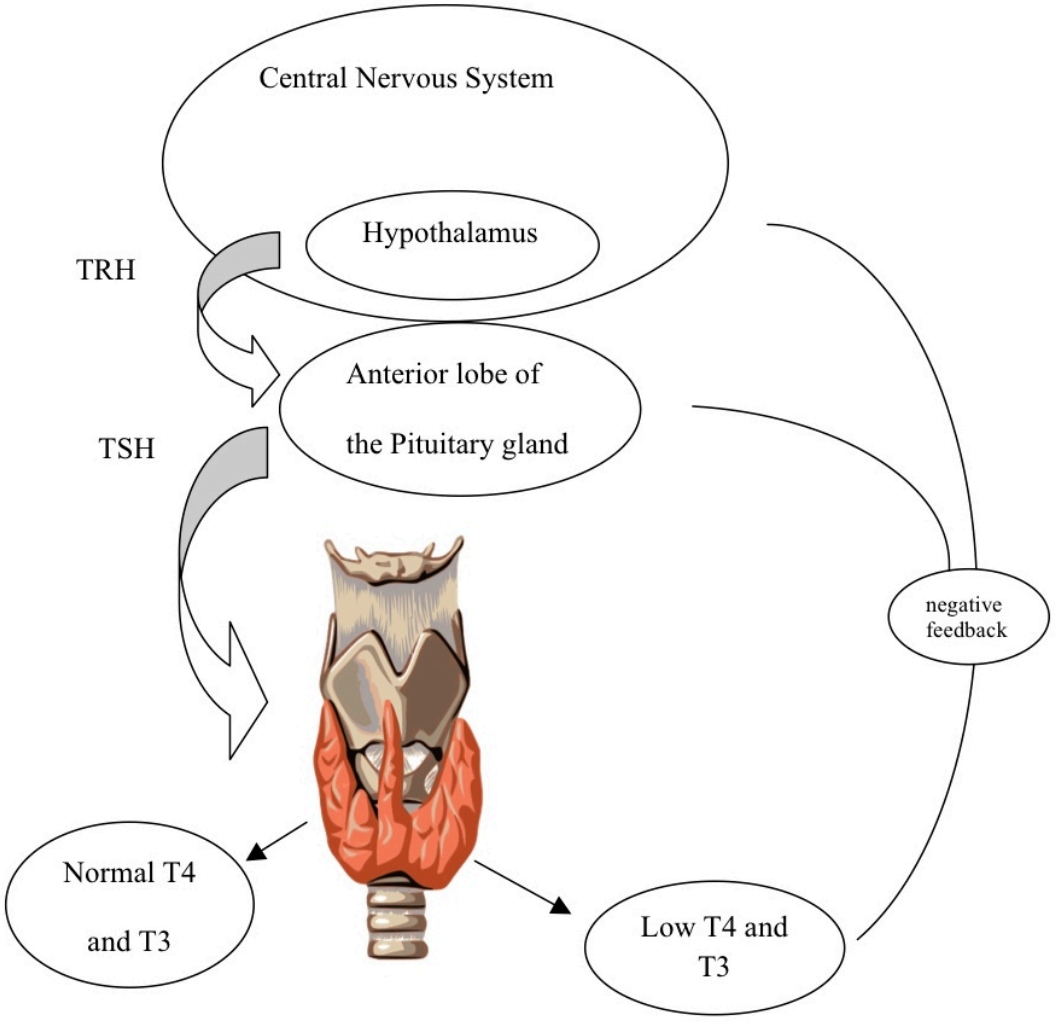

Figure 1-2 Mechanism of the thyroid hormones regulation.

Thyrotropin releasing hormone (TRH) in the hypothalamus is activated by the negative feedback of low thyroxin (T4) and triiodthyronin (T3) in the circulation. Secretion of TRH stimulates the anterior lobe of the pituitary gland to produce thyroid stimulating hormone (TSH). TSH stimulates the thyroid gland; synthesis and secretion of T4 and T3 hormones are then compensated.

1.3.2 Thyroid hormones and pregnancy

Although increased demand for energy, nutrients, minerals and vitamins is usually a benign physiological feature of pregnancy, biochemical values of the thyroid function can still be altered (Burrow, 1990, Glinoer, 1999, Glinoer, 2004). Pregnancy has been described as a status where dramatic hormonal changes take place that result in increased thyroxin binding globulin (TBG), an increase in urinary iodine excretion, increased activity of the placenta, decreased TSH and increased human chorionic gonadotropin (HCG) (Glinoer, 1999). This results in increased thyroid hormones, metabolism and an increased demand for iodine (Glinoer, 2004, Yassa et al., 2010). During the first and second trimesters, level of TBG and T4 increase under the effect of increased levels of estrogens and HCG, respectively (Epstein et al., 1994, Glinoer, 1999). Maintaining the balance between these changes and normal progressing of the pregnancy is achievable in iodine sufficient area, difficult in iodine insufficient areas (Glinoer, 2004).

1.3.2.1 Role of iodine and thyroid hormones in brain development

The relationship between the thyroid gland, severe iodine deficiency and neurological manifestations in human (e.g. cretinism) was first established and documented in the literature (Cranefield, 1962). During foetal development, the thyroid gland was found to reach maturation at 11-12 weeks of gestational age (Obregon et al., 2007). The presence of thyroid hormones in foetal neurological tissues was evidenced by the detection of T4 and T3, it was suggested that T4 and T3 play a crucial role in growth and neuropsychological development during and after gestation (Chan and Kilby, 2000, Koibuchi and Chin, 2000, Calvo et al., 2002, Kester et al., 2004, Patel et al., 2011). It was also explained earlier that myelination

and neuronal/glial cell differentiation is regulated initially by thyroid hormones at genes level (Bernal and Nunez, 1995).

1.4 Thyroid diseases and pregnancy

Goitre is one of the prominent sign of thyroid disease in iodine deplete regions (Andersson et al., 2007, Zimmermann, 2010). While hypothyroidism, as result of goitre is believed to be more common in iodine-poor regions, an autoimmune-origin of thyroid diseases (e.g. Grave's disease and Hashimoto thyroiditis) were found to be the most common causes in iodine-replete areas (de Benoist et al., 2003, Andersson et al., 2007, Vanderpump, 2010).

Prevalence of thyroid dysfunction can vary from as low as 0.1 % to as high as 15.5% (Table 1-1). It was reported by some large-scale studies that the prevalence of thyroid disease during pregnancy, identifying 0.3-0.5% of overt hypothyroidism (OH) cases and 1.5-4% of subclinical hypothyroidism (SCH) (Allan et al., 2000, Casey et al., 2005, Negro and Mestman, 2011). Iodine deficiency was found to play a negative role in thyroid function, it was emphasised that a higher percentage of disease to be anticipated more in iodine-deplete areas (Klein et al., 1991, Stagnaro-Green et al., 2011). Thus, OH and SCH, are the two disorders most commonly encountered thyroid disorders during pregnancy in iodine poor areas and consequently believed to be associated with adverse effects on the development of the foetus and on the course of the pregnancy (Stagnaro-Green et al., 2005).

Table 1-1 TSH upper normal value and prevalence of thyroid dysfunction

Source	**TSH**	**Prevalence / comments**
(Hollowell et al., 2002)	4.5	4.3% SCH, 0.3% OH
(Haddow et al., 2004)	1st trimester >5.2 2nd trimester >4.2	4% Hypothyroidism
(Casey et al., 2005)	>97.5th percentile	2.5% SCH, 0.2% OH
(Vaidya et al., 2007)	4.2	1.6% SCH, 1% OH
(Marwaha et al., 2008)	4.2	14.2% SCH, 1.3% OH
(Gilbert et al., 2008a)	2.15	4.5% elevated TSH
(Blatt et al., 2012)	Trimester specific	15.5% tested positive for gestational hypothyroidism
(Moreno-Reyes et al., 2013)	1st trimester 2.5-3 2nd trimester >3	7.2% (6.8% SCH, 0.4% OH)
(Altomare et al., 2013)	1st trimester 2.5-3 2nd trimester >3	12.3% had high TSH (hypothyroidism)
(Diéguez et al., 2016)	4.5	5.5% (1.9% OH, 3.6 %SCH)

As pregnancy has been reported to affect thyroid function, monitoring for thyroid activity using certain laboratory parameters has been proposed (Glinoer, 1999, Glinoer, 2004). Upon conception, hCG hormone increases which in turn stimulate thyrocytes (TG) to induce a transient increase in FT4 levels, which is mirrored by a lowering of TSH concentrations. Following this period, serum FT4 concentrations decrease slightly (10–15% on average), and serum TSH values steadily return to normal. In line with these variations, both FT4 and TSH reference intervals change throughout pregnancy, depending on gestational age (Vermigllo et al., 1995, Glinoer, 2004). This is generally stabilised by the end of the second trimester when iodine intake was adequate (Glinoer, 2004). Thus, trimester specific TSH reference was highly recommended (Table 2-1). The table demonstrates variability in the intervals from one population to another. Therefore, developing population sample-based trimester-specific range for TSH is mandatory.

T3/T4 ratio is another parameter found to reflect thyroid stimulation during pregnancy (Glinoer, 1999). The last parameter proposed for monitoring thyroid behaviour during pregnancy was TG, it was reported to have tendency to elevate more in the third trimester of pregnancy (Rasmussen et al., 1989, Glinoer, 1999). Although measuring FT4 using liquid chromatography/tandem mass spectrometry (LC/MS/MS) methods have been recommended, using trimester-specific TSH has been advised to consider as well (Stagnaro-Green et al., 2011).

Thyroid diseases with an autoimmune association, is the most prevalent in iodine sufficient regions (Moreno-Reyes et al., 2013). It was reported that 10-15% of iodine sufficient population tested positive to thyroid peroxidase (TPO) antibodies (Glinoer

et al., 1994, Pop et al., 1995). The presence of TPO antibodies have been linked to pregnancy complications, postpartum thyroid dysfunction and progression to a symptomatic disease (Negro et al., 2011). Furthermore, the risk of spontaneous abortion and preterm delivery were also increased in women with auto antibodies (Glinoer et al., 1994). Evidence of complications related to positive thyroid autoimmune status of pregnant women living in iodine sufficient conditions are far greater than of negative TPO status. Nevertheless, testing for thyroid antibodies are found to be of very little clinical help because a clear relationship between these antibodies and recurrent pregnancy loss could not be established (Negro et al., 2010b).

Table 1-2 Population-based trimester specific intervals for TSH

	Trimester[a]		
Reference	**First**	**Second**	**Third**
[1] (Haddow et al., 1999)	0.08-2.73	0.39-2.70	_
[2] (Stricker et al., 2007)	0.09-2.83	0.20-2.79	0.31-2.90
[3] (Panesar et al., 2001)	0.03-2.30	0.03-3.10	0.13-3.50
[4] (Soldin et al., 2004)	0.24-2.99	0.46-2.95	0.43-2.78
[5] (Bocos-Terraz et al., 2009)	0.03-2.65	0.12-2.64	0.23-3.56
[6] (Marwaha et al., 2008)	0.60-5.00	0.43-5.78	0.74-5.70
[7] (Blatt et al., 2012)	0.10 - 2.50	0.55 - 2.75	0.43 - 2.91
[8] (Diéguez et al., 2016)	0.20- 4.50	_	_
(Gilbert et al., 2008a)	0.02–2.15	_	_

[a] TSH in mIU/L, with parenthetical data indicating 5th and 95th percentiles (1,3,6) or 2.5th and 97.5th percentiles (2,4,5,7,8)

1.4.1 IDD Complications in pregnancy

Maternal, foetal and neonatal complications of untreated thyroid diseases during pregnancy have been well investigated (Cranefield, 1962, Davis et al., 1989, Millar et al., 1994, Haddow et al., 1999, Allan et al., 2000, Glinoer, 2001, Abalovich et al., 2002, Casey et al., 2005, Casey et al., 2006, Casey and Leveno, 2006, Negro et al., 2011, Sarkar, 2012, Männistö et al., 2013). Although complications due to thyroid dysfunction during pregnancy can widely be variable, some conditions such as (gestational diabetes, gestational hypertension, miscarriage, preterm delivery and foetal death) were frequent outcome (Table 3-1).

1.4.1.1 Obstetric complications

Untreated OH was demonstrated to show negative impact on pregnancy outcome as well as foetal neuropsychological development (Abalovich et al., 2002, Haddow et al., 1999). Risk of foetal loss was reported to be as high as 60% among pregnant women with OH (Abalovich et al., 2002). It was also found that the risk for gestational hypertension increases to 22% in pregnant women with OH compared to those without (Leung et al., 1993). It was concluded that risk of foetal death was found to be increased among a study population with combined OH and pregnancy (Allan et al., 2000). Although association between GDM and hypothyroidism have previously been rejected, later publication reported a positive relationship (Casey et al., 2007, Cleary-Goldman et al., 2008, Korevaar et al., 2013).

Placental abruption and preterm delivery were demonstrated to be three times and 1.8 times, respectively more likely in women with SCH. Preterm deliveries (<32weeks)

had a three-fold incidence in subclinical hypothyroidism (Casey et al., 2005, Stagnaro-Green et al., 2005). A recent study has shown that pregnant women with SCH are at significant risk of developing gestational diabetes (Negro and Stagnaro-Green, 2014). Also, the risk of spontaneous abortion and preterm delivery were increased in women with auto antibodies (Glinoer et al., 1994, Glinoer, 2001).

1.4.1.2 Neonatal complications

Neonates also share risk of complications with maternal thyroid disease with the birth weight of children born to mothers with OH found to be decreased (Idris et al., 2005). Although guidelines for early diagnosis and management of overt hypothyroidism in pregnancy has been well established, complications such as congenital hypothyoiridism, neuropsychological development (IQ) deficit and foetal death are still prevalent in some countries (Blazer et al., 2003, Chan and Kilby, 2000, de Escobar et al., 2004).

It was suggested that untreated hypothyroidism and probable subclinical hypothyroidism during pregnancy was associated with a risk of a poorer outcome and a three-fold increased predisposition for learning difficulties (Haddow et al., 1999). Nevertheless, a study conducted in the Netherlands failed to define any association between thyroid status in early pregnancy and cognitive development in offspring (Henrichs et al., 2010). Hypothyroxinemia detected during the first trimester, irrespective to the increased TSH, associated with a higher risk of poor neuropsychological development of the progeny (Morreale de Escobar et al., 2000). In contrast, other studies could not find positive relationship between maternal

hypothyroxinemia and impairment of neonatal cognitive development (Oken et al., 2009, Craig et al., 2012).

Further studies reported that neonatal sepsis, respiratory distress syndrome (RDS), transient respiratory tachypnoea and apnoea were associated with maternal thyroid disorders, either primary hypothyroidism or hyperthyroidism (Männistö et al., 2013). More recently, smaller head circumference and lower brain weight were found being linked to babies born to TPO positive mothers who were white/non-Hispanic (Wilson et al., 2014).

Table 1-3 Variability of maternal and neonatal complications in association with thyroid dysfunction

Reference	**Type of thyroid dysfunction**	**Maternal complications**	**Neonatal complications**
(Toulis et al., 2014)	Subclinical hypothyroidism (SCH)	Gestational diabetes (GD)	
(Korevaar et al., 2013)	SCH Isolated hypothroxinemia (IH)	Premature labour (PL) 2.5-fold increased risk of premature delivery, a 3.4-fold increased risk of spontaneous premature delivery, and a 3.6-fold increased risk of very premature delivery	
(Mannisto et al., 2013)	Subclinical hypothyroidism(SCH)	Increased risk for GD, Pre-eclampsia Preterm	Preterm babies, sepsis, ARDS
(Karakosta et al., 2012)	SCH+Autoimmunity	4-fold increase risk for GD	3-fold increase risk for low birth wt.
(Tudela et al., 2012)	SCH	3-fold in GD	
(Stagnaro-Green, 2011)	Overt hypothyroidism(OH)	Pre-eclampsia,gestational hypertension(GHTN) and spontaneous abortion.	cretinism, foetal death
(van den Boogaard et al., 2011)	SCH	Pre-eclampsia	
(Su et al., 2011)	OH SCH		-Increased fetal loss, low birth weight, and congenital circulation system malformations. -Foetal distress, preterm delivery, poor vision development, and

	IH		neurodevelopment delay. -Foetal distress, small for gestational age, and musculoskeletal malformations.
(De Vivo et al., 2010)	SCH Autoimmunity	Increased risk for miscarriage Increased risk for miscarriage	
(Ashoor et al., 2010)	SCH	Increased risk for miscarriage	
(Negro et al., 2010a)	SCH with TPOAB	Increased risk for miscarriage	
(Li et al., 2010)	SCH		Impaired neuropsychological development
(Benhadi et al., 2009)	SCH		Foetal death
(Vermiglio et al., 2004)	IH with iodine deficiency		Impaired neurosychological development(attention deficit and hyperactivity disorder)
(Allan et al., 2000)	SCH (TSH>6.0mIU/L)		Foetal death
(Leung et al., 1993)	SCH Isolated hypothyroxinemia(IH)	GHTN, pre-eclmpsia Preterm labour, GD	Macrosomia

1.5 Iron

Iron has been described as a component of metaloproteins that are essential for biochemical activities; oxygen sensing and transport, electron transfer and catalysis (Papanikolaou and Pantopoulos, 2005). In the human body iron content was reported to vary between 3 and 5 grams, 70% of which found being utilised for heme and hemoglobin synthesis (Ponka et al., 1998). The liver, spleen, bone marrow, muscles, the kidneys and macrophages of the reticular-endothelial system are other organs where iron is being utilised and stored (Bernát, 1983, Siah et al., 2006).

Deoxidising the alimentary non-heme (Fe^{3+}) iron to heme (Fe^{2+}) form, is an essential process to facilitate iron absorption in the duodenum (Figure 1-3) (Pantopoulos, 2004). Whereas there no direct mechanism for iron excretion expressed in mammals, the normal physiological balance of iron is maintained by dietary compensation for the normal loss that occurs by desquamation of epithelial cells from the gut, skin, and genitourinary tract (Bothwell et al., 1979).

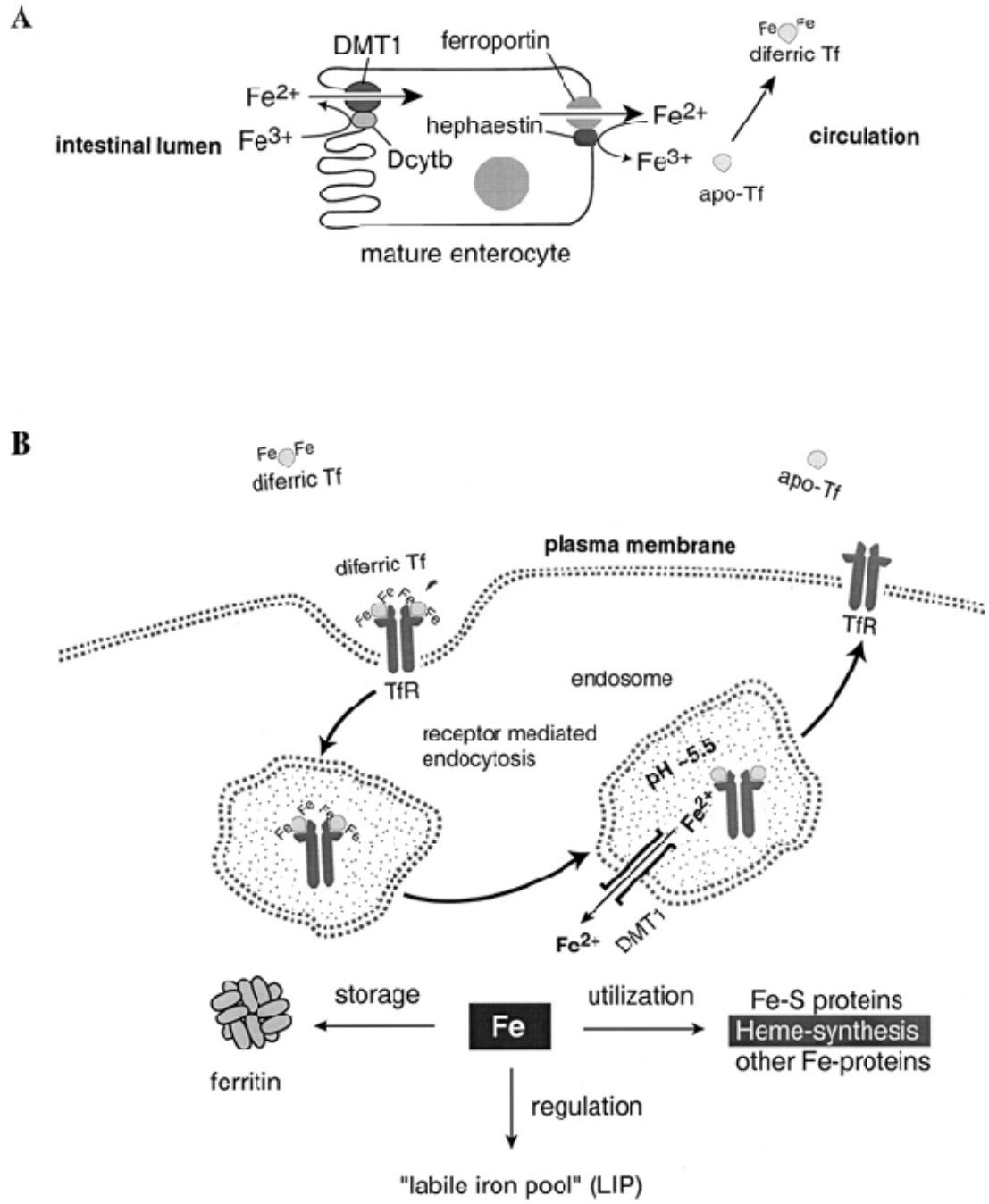

Figure 1-3 Overview of iron transport and metabolism

(A) Iron transport across duodenal epithelial cells. Reductase of the deudenal cytochrom-b (Dcytb) change ferric iron into ferrous iron. The divalent metal transporter (DMT1) then transport Ferrous iron across the apical membrane of the mature enterocyte. Iron then exported to the circulation by the ferroportin. This step is coupled with reoxidation of ferrous to ferric iron by membrane-bound hephaestin. Plasma iron is immediately scavenged by transferrin (Tf). (B) Schematic representation of the Tf cycle. Plasma Tf binds to its cell surface receptor (TfR) and the complex is internalized by endocytosis. Acidification of the endosome results in the release of ferric iron from Tf and reduction and subsequent transport of ferrous iron across the endosomal membrane by DMT1. The pathway is completed by recycling of the apoTf-TfR complex to the cell surface and release of apoTf. Intracellular iron is utilized for the synthesis of iron-containing proteins and the excess is stored into ferritin. A fraction of chelatable iron defined as “labile iron pool” (LIP) determines the cellular iron status (Pantopoulos, 2004)

1.5.1 Iron requirement

During pregnancy the foetus stores approximately 250 mg of iron that is drawn on during breastfeeding (Fomon, 1993). While daily recommended requirement for absorbed iron is 0.55 mg per day, breast milk supplies only 0.15 mg of absorbed iron per day (Fomon, 1993). During child growth 0.5 mg of iron per day is absorbed in excess of body losses; adequate amounts of iron during growth typically results in a 70-kg man accumulating about 4 g of body iron (Fomon, 1993). Due to menstrual blood loss women generally have less iron stores than men and requirement for iron in a healthy childbearing-aged population estimated to be approximately 1.5-2 g per day (Bothwell et al., 1979, Andrews, 1999).

Although iron bioavailability (absorption) is affected by diet, pregnant women during the second trimester are still recommended to take >50mg/d regardless of the type of diet. Despite the fact that there is little evidence to support detrimental maternal or foetal outcomes, advocating for universal iron supplementation of pregnant women has been carried out in the industrialised countries (Zimmermann and Hurrell, 2007). A prenatal iron supplementation in iron-replete, non-anaemic low-income pregnant women in the USA was found to decrease incidence of preterm delivery and increase birth weight (Cogswell et al., 2003, Siega-Riz et al., 2006).

1.5.2 Iron Deficiency Anaemia (IDA) during pregnancy

Chlorosis or “the green sickness” was the term used in the medieval age describing people with pallor (Guggenheim, 1995, Kiple and Ornelas, 2001). Approximately 24.8% of the world’s population is affected by anaemia, 30-40% of the cases were

pregnant women and preschool children (Benoist et al., 2008, McLean et al., 2009). It has been reported that 75% of anaemia in pregnancy has been identified to be of iron deficiency type (Benoist et al., 2008, McLean et al., 2009, Horowitz et al., 2013). It was also reported that heavy menstruation, high parity, intrauterine devices and vegetarian diet were strong contributing factors on the rise of IDA (Zimmermann and Hurrell, 2007). In Northern Tasmania 20% of pregnant women have been identified to be suffering from IDA (Khalafallah et al., 2010).

Pregnancy has previously been explained to be a condition when foetal growth and maternal blood volume expansion lead to increased nutritional demand including iron (Hallberg, 1995). It was established that iron requirement during pregnancy was equal to that contained in about 4 units of blood (about 1 kg), most of which is needed in the last two trimesters (Bothwell et al., 1979).

From a dietary aspect IDA was highly expected when iron requirements are greater than energy needs and that would be the case in infants and young children, adolescents, heavily menstruating women and pregnant women (Earl and Wotecki, 1993, Zimmermann and Hurrell, 2007). It was found that women with multiple pregnancies were susceptible to IDA as well as their offspring when there were no enough intervals between pregnancies for the body to re-establish it's iron stores (Kilbride et al., 1999).

1.5.3 Possible complications of IDA in pregnancy

Although there is not conclusive evidence regarding the effect of maternal IDA on infants, cognitive and motor development in school aged children have been shown

to be affected (Scholl and Hediger, 1994, Sachdev et al., 2005). IDA was found to increase the risk of preterm labour, low birth weight and infant mortality particularly during the first and second trimesters of pregnancy (Brabin et al., 2001).

It was suggested that children who have IDA in infancy are at risk of delayed developments when compared to those with normal iron status (Lozoff et al., 1991). In contrast, adverse effects of iron deficiency and/or the absence of iron in the developing brain on neuromotor development were not conclusive (Beard, 2001).
IDA in third trimester and its effect on child's mental development were studied in China, resulted in a significant lower mental development index (Chang et al., 2013). Susceptibility to/and duration of infections in children, mainly of the upper respiratory tract was reported to be aggravated by IDA (de Silva et al., 2003).

1.5.4 IDA and thyroid disease

The association between thyroid diseases and anaemia was first reported in the late 19th century when post-thyroidectomised patient developed anaemia, although the reasons for this were unclear (BOMFORD, 1938). It was reported that 53/202 (26.2%) of patients pre-diagnosed with hypothyroidism had anaemia (Horton et al., 1976). Furthermore, 60/118 (50.8%) of patients who were studied more closely, were found to have iron level at the lower margin of the reference interval (<12 µmol/L) and 42 (35.5%) had a low serum concentration of iron (Horton et al., 1976).

Using haemoglobin only to evaluate IDA was labeled as non-sensitive and non-specific (Zimmermann, 2008). Although serum ferritin was reported to be a better parameter in reflecting iron store, it found to be unreliable in the presence of active

inflammatory process. Iron saturation transferrin and erythrocyte zinc protoporphyn were used to determine iron-deficient erythropoiesis, but lacking accurate diferentiating acute IDA from anaemia of chronic illness was its back-draw(Zimmermann, 2008). Hence, a combination of serum ferritin and serum transferring receptor was suggested for evaluating iron store and tissue iron deficiency, respectively (Zimmermann, 2008) .

IDA has also been found to have a negative impact on maternal thyroid function, which is compounded in areas where there are nutritional deficiencies (Zimmermann et al., 2000). In regions of endemic goitre, thyroid response to iodine supplementation was impaired in children with IDA compared to iron sufficient children (Zimmermann et al., 2000). In another study, the efficacy of administered iodised salt was improved in children suffering from goitre, by managing IDA (Hess et al., 2002). This is supported by another study demonstrating that combined supplementation with Fe and Iodine had even better effect on thyroid function than iodine supplementation alone (Eftekhari et al., 2006).

1.6 Screening for IDD in pregnancy

Regardless of the growing evidence of thyroid disorders and its consequences on pregnancy, foetal growth and neuropsychological development of children, clinicians have been reported to still prefer a case finding approach rather than generalised screening for thyroid diseases (Glinoer, 2001, Casey et al., 2005, Casey and Leveno, 2006, Casey et al., 2006, Vaidya et al., 2007). It has been suggested that 20-50% of pregnant women with suspected thyroid disease would be missed when selectively approached for thyroid screening (Vaidya et al., 2007, Horacek et al., 2010).

A recommendation in favour of generalised screening for thyroid diseases during pregnancy was made on a joint statement by the American Thyroid Association (ATA), the American Association of Clinical Endocrinologists (AACE) and the Endocrine Society (ES) in 2005 (Gharib et al., 2005). In a subsequent meeting the previous statement has been changed, universal screening of thyroid diseases was not as effective as a selective screening approach (Stagnaro-Green et al., 2011, Garber et al., 2012).

In contrast, some European countries have introduced universal screening for thyroid disease during pregnancy, an approach that has been widely accepted, particularly in Spain and Belgium (Vila et al., 2014). After a thorough review of the serious impact of undiagnosed and therefore untreated OH on the mother and foetus, and neurocognitive development of the unborn child, universal screening has been strongly suggested (Stagnaro-Green, 2012).

General screening has been found to be cost effective not only when compared with no screening, but also when compared with screening of high-risk women (Thung et al., 2009, Dosiou et al., 2012). Although, the latest conjoint statement made by the American thyroid association don't recommend a general screening of the thyroid diseases in pregnancy, disagreement and consequences of late diagnosis and/or untreated cases have been emphasised by others (Allan et al., 2000, Abalovich et al., 2002, Gharib et al., 2005, Vaidya et al., 2007, Stagnaro-Green et al., 2011).

1.6.1 Screening tools and interpretation of results

1.6.1.1 Screening for maternal thyroid function

Testing for TSH, FT4, FT3 and TPO antibodies are basically required in order to assess thyroid function. Most biochemical tests are performed using sophisticated automated devices in the biochemistry sections of medical laboratories. Immunoassay is the most common method for measuring FT4 and FT3, reliability of which during pregnancy has been reported to be questionable (Lee et al., 2009). Chemiluminescent (immunoassay-based) can be refered to substances capable of yielding higher specific activities when used as direct reagent labels in this context, and thus provide a basis for the development of 'ultra-sensitive', non-competitive, immunoassay methodologies (Ekins et al., 1989). Enzymes catalysing chemiluminescent reactions or yielding fluorescent reaction products can likewise be used as labels yielding high effective specific activities and hence enhanced assay sensitivities (Ekins et al., 1989).

It has been reported that the serum of pregnant women is characterized by higher level of TBG and nonesterified fatty acids and by lower concentrations of albumin compared to the serum of non-pregnant women (Stagnaro-Green et al., 2011). One of the most sophisticated devices is the dialysate or ultrafiltrate online solid phase extraction–liquid chromatography/tandem mass spectrometry (LC/MS/MS), it has been highly recommended for accurate measurement of FT4 (Yue et al., 2008, Stagnaro-Green et al., 2011). In addition, it was demonstrated that LCMSMS enhanced diagnostic capabilities by affording the specificity, precision, and limiting of quantification necessary for the reliable measurement of thyroid hormones. These methods were found to be especially important in states of disease and during

pregnancy when protein binding is a factor that interferes with other methods for thyroid hormone analysis (Soldin and Soldin, 2011, Hoofnagle and Roth, 2013).

Regardless previous recommendation it has been reported by other authors that immunoassay performs well, show low results when expected and report high results when excess of FT4 occurs (Anckaert et al., 2010). Unavailability of LC/MS/MS prompts each laboratory individually estimated trimester-specific references for TSH has been recommended to follow instead (Stagnaro-Green et al., 2011). In case laboratories don't have their own trimester-specific reference for TSH, the following reference ranges have been recommended to follow; first trimester, 0.1–2.5 mIU/L; second trimester, 0.2–3.0 mIU/L; third trimester, 0.3–3.0 mIU/L (Stagnaro-Green et al., 2011). It is worth mentioning that LGH pathology (where study samples were processed) reports their reference range for TSH and FT4 in the general population as (0.47-4.70 mU/L) and (10.0-28.2 pmol/L), respectively.

1.6.1.2 Screening for iodine status

As more than 90% of ingested iodine was found to be excreted by kidneys, urinary iodine (UI) was thought to be the best source for iodine level evaluation (Vought and London, 1967). While UI 24 hour urine was described to be a better indicator, UI can be measured in spot urine specimens from a representative sample of the target group and expressed as the median, in µg/L (World Health Organization, 2007). For national school-based surveys of iodine nutrition, the median UI from a representative sample of spot urine collections from approximately 1200 children (30 sampling clusters of 40 children each) can be used to classify a population's iodine status, according to WHO (World Health Organization, 2007).

Although the median UI does not provide direct information on thyroid function, a low value suggests that a population is at higher risk of developing thyroid disorders (Zimmermann, 2009). It was argued that median UI's variability from day to day made it an easy subject for misinterpretation (Andersen et al., 2008). It was further explained that assuming subjects as iodine deficient with a spot UI of less than 100 μg/L is not acceptable (Zimmermann, 2009). Since 24 hour urine collection was not practical for large scale screening also to avoid day-to-day variability in spot UI, use of age- and sex-adjusted (iodine:creatinine) ratio in adults was thought to be a better alternative (Knudsen et al., 2000). Using the following formula; urinary iodine (μg/L) × 0.0235 × body weight (kg) = daily iodine intake, a median UI of 100 μg/L corresponds roughly to an average daily intake of 150 μg.

Currently, UI estimation varies according to the level of technology it is associated with. Thus, Urinary Iodine in this study was measured directly by ICPMS following dilution in an ammonium EDTA based diluent using rhodium as an internal standard (Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer).This analysis was developed at the Biochemistry Department at the Royal Prince Alfred Hospital, Sydney. Inductively coupled plasma mass spectroscopy (ICP-MS) is an analytical technique that is capable of performing quantitative multi-element analysis at trace concentrations of these elements. An ICP-MS is capable of measuring concentrations to a level of ng / L of the analyte. Nevertheless, most modern techniques for testing UI are originally based on the old basic principles (the Sandell-Kolthof reaction) (World Health Organization, 2007, World Health Organization, 2013). Using the median urinary iodine concentration, criteria was developed by the WHO to assess

iodine intake in target groups. According to results of median UI (μg/L) for pregnant women, iodine intake was evaluated; UI < 50 μg/L would indicate to insufficient iodine intake (World Health Organization, 2013). While 150-249 μg/L would describe adequate iodine intake, 500 μg/L and more would be evaluated as excessive level (World Health Organization, 2013). Lactating women were categorised differently bearing in mind iodine excretion in milk as well as in urine; median UI < 100 μg/L would be indicating insufficient intake and a 100 μg/L and more would be of adequate intake (World Health Organization, 2013).

1.7 Cord blood and neonatal thyroid assessment

Umbilical cord blood has long been endeavoured for evaluating neonatal thyroid status (Walfish et al., 1979, Henry et al., 2000). Maternal and neonatal hypothyroidism has a negative impact on the foetal neuropsychological development (Rovet et al., 1987, Haddow et al., 1999, Idris et al., 2005). Although a well correlation was reported between TSH results of the umbilical cord blood and the neonatal venous blood, the latter have been recommended (Walfish et al., 1979, Jacob and Peters, 2015). Since confirmatory venous blood TSH was required for elevated cord blood TSH results, recalling the patients back demonstrated to be a non-practical approach (Walfish et al., 1979, Wu et al., 1999).

1.8 Aims and Hypothesis

Aims:

1. To biochemically, determine the rate of thyroid function alterations within a limited group of obstetric population in northern Tasmania.
2. To determine whether altered thyroid was associated with clinical complication to the mothers and their babies during pregnancy and delivery.
3. To determine whether iron deficiency anaemia and iodine deficiency are associated with low thyroid status.
4. To find out whether a general screening of thyroid diseases for pregnant women, identify more cases than the current routine selective screening.
5. To determine if cord TSH can be used as a screening tool for neonatal hypothyroidism.

Hypotheses:

1. The number of pregnant women with thyroid disorder can be more significant if biochemically screened either prior conception or at early pregnancy.
2. Thyroid function disorder during pregnancy has a negative impact on maternal and foetal wellbeing.
3. Iodine deficiency and low ferritin levels are possible predictors for the thyroid dysfunction.
4. General biochemical screening for thyroid function is a better approach in identifying thyroid diseases during pregnancy and preventing associated complications.
5. Cord blood TSH, a non-invasive tool for neonatal hypothyroidism screening.

2 MATERIALS AND METHODS

2.1 Study Design

2.1.1 Ethics

The study was approved by the Tasmanian Health and Medical Human Research Ethics Committee (H119759) and was conducted between 16/04/2012 and 15/04/2014 at the Launceston General Hospital (LGH), Tasmania, Australia. Informed consent was obtained by all participants.

2.1.2 Recruitment

Since all pregnant women went through pathology for gestational diabetes screening, thinking of the pathology as a capturing point was foreseeable. Hence, potential participants were invited to take part in the study while they were waiting at the pathology for glucose tolerance test (GTT). Upon their interest and prior to consent for participation, all women were individually informed about the project and possible outcomes. Pregnant women aged 18 years and above with absence of any known history of thyroid disease were included in the study (Figure 2-1). The researcher documented personal information, demographic data, medical history and medication profiles. Participants with abnormal laboratory results (high TSH levels) were contacted, the condition explained and referred onto the endocrinologist. It is worth mentioning that recruitment and sample collection was happening simultaneously.

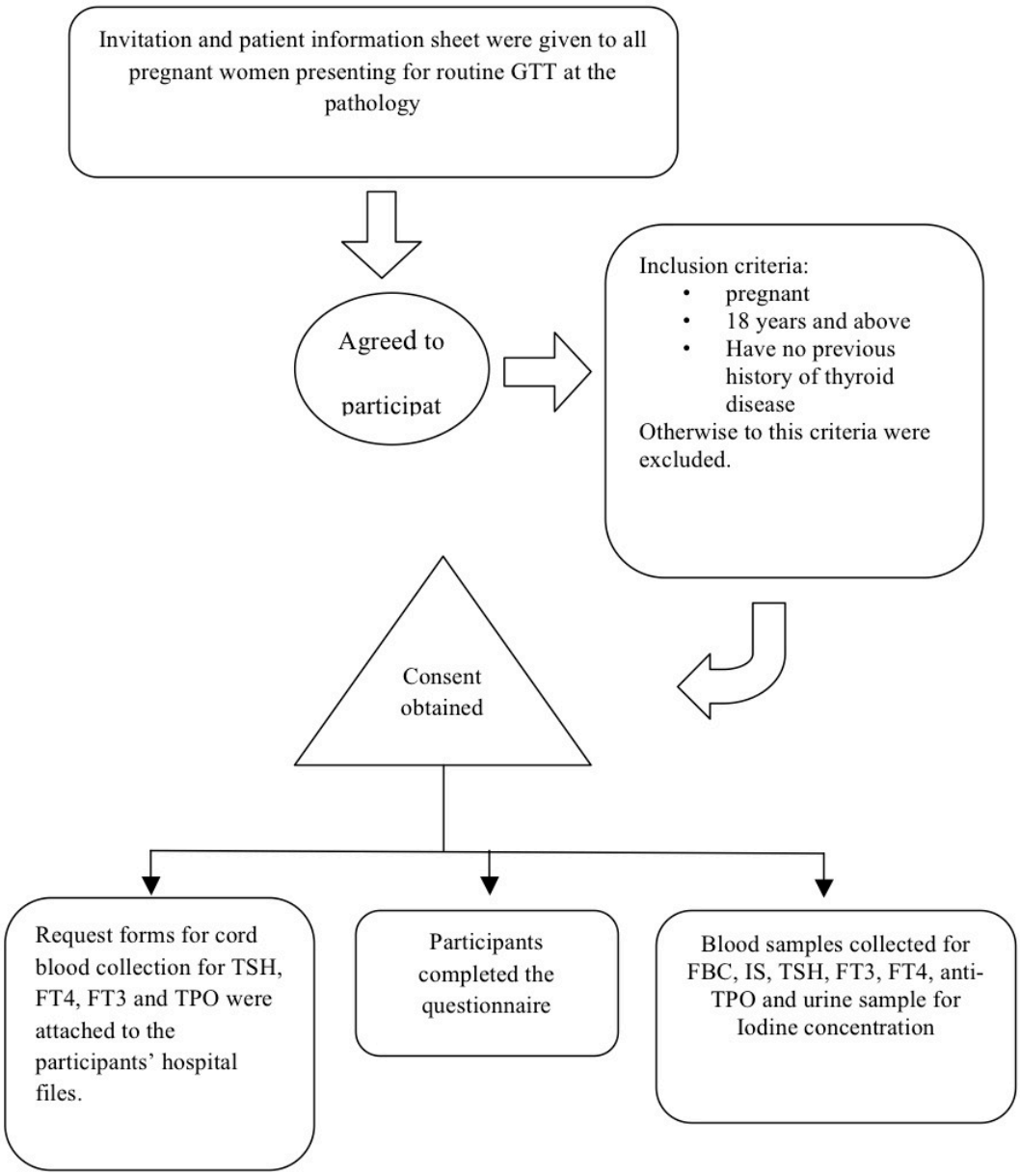

Figure 2-1 Study design and Recruitment flow

First stage, at pathology reception where invitation and information sheet were handed out to all pregnant women presented for GTT. Second stage, inclusion/exclusion criteria were applied to the interested women and informed consents were obtained. Third stage, a questionnaire was filled by the participant and blood nurses were notified to collect the samples.

Data capturing sheet was derived and modified from Abalovich *et al* 2002 and their recommended approach for case finding. The data capturing sheet was instituted in two parts; the first part recorded physical parameters (weight and height) and visible signs of goitre (enlarged thyroid gland, exophthalmia and pretibial oedema) as assessed by the study investigator. Gestational age, expected date of delivery, and type of conception were also documented.

Using thyroid function testing markers (TSH, FT4 and FT3) was set as a screening tool (predictors) for the study group. Hence, this will divide the study population into normal, low thyroid status (TSH>2.5mU/L) and isolated low thyroxin (FT4<8.9pmol/L). Likewise, cord blood samples were also collected as part of the study from the participants' babies and were tested for TSH, FT4, FT3 and anti-TPO antibodies. In addition to that, full blood count (FBC) and iron studies (IS) were part of the investigations. In addition, urine samples were collected from the participants for measuring urinary iodine (UI) level.

The second part was the questionnaire form, which was purposely designed to obtain specific clinical information to identify women who would have been candidates for thyroid testing based on history and symptoms alone. It includes past and present medical history, obstetrics & gynaecological history, medications, dietary history and symptoms of hyperthyroidism or hypothyroidism. All abnormal findings were documented by the investigator and discussed with the principal investigator for further investigations as required. This may include referral for thyroid ultrasound or to an endocrinologist if clinically required. Participant's GP's were notified with any abnormality and action plan.

Furthermore, records of maternal, foetal and neonatal complications were collected in order to determine the incidence rate in each group of thyroid status. Clinical outcomes in the study cohort are divided into two groups; antenatal (maternal/foetal) complications and neonatal wellbeing parameters.

Ante-partum complications are defined by the presence of the following parameters: oligohydromnious, polyhydromnious, gestational diabetes mellitus (GDM), gestational hypertension (GHTN) and pre-eclampsia, infection, placenta previa, threatened premature labour (TPL), ante-partum haemorrhage (APH), intra-uterine foetal growth restriction (IUFGR) and organ anomalies, macrosomia, biliary disease and abdominal or pelvic surgery. Intra-partum complications are defined by the presence of the following: cord prolapsed, prolonged labour, instrumental delivery, intrapartum haemorrhage (IPH), pyrexia and hypertension (HTN). Post-partum complications are defined by the presence of postpartum haemorrhage (PPH) and wound infection. Neonatal parameters include Apgar scores, head circumference, resuscitation and nursery admission.

Once the questionnaire was completed, participants were given a copy of the signed consent with two pathology forms (Antenatal) for blood and urine samples. Antenatal pathology forms requested collection of blood and testing for TSH, free thyroxin (FT4), free triiodthronine (FT3), and anti-TPO antibodies as well as urine sample for iodine level. IS and a FBC were also performed to test for the presence of concomitant IDA. A cord-blood pathology form was attached to a copy of the consent of each participant and placed in the participant's medical record.

Participants' medical records were also marked with a pink-coloured label in order to help the labour ward nursing staff identify the participants easily.

2.2 Sample collection and handling

Fasting blood (10 ml) and urine (15 ml) specimens were collected from consented participants during daytime working hours. Blood samples were collected into different tubes according to the requested test. Specimens for FBC were collected in purple top BD Ethylenediaminetetraacetic acid (EDTA)-Vacutainer (K2E) tubes (Becton Dickenson and company, Franklin Lakes, NJ, US). Blood for TSH, FT4, FT3, anti-TPO antibodies and IS were collected in gold top BD Vacutainer (SST II) tubes. Similarly, cord blood samples for TSH, FT4, FT3, and anti TPO-antibodies were collected in gold top BD Vacutainer (SST II) tubes. Urine samples for iodine measurement were collected in plain sterile containers.

Blood samples for FBC were tested within 30 minutes of collection, while samples for TSH, FT4, FT3 and anti-TPO antibodies were centrifuged at 4000 x g for five minutes and one aliquot of serum were forwarded to the Biochemistry laboratory for routine testing for TSH, FT4, FT3, while another 2-3 aliquots are stored at -70^0 C.

Urine samples were divided equally between two conical tubes and paired with anti-TPO antibody samples, then stored at -70^0C. Approximately one hundred frozen samples were collected and transported to the Royal Prince Alfred (RPA) Hospital, Pathology, Sydney for analysis. Cool boxes with dry ice were used to keep the sample temperatures at -70^0C during interstate transportation.

2.3 Tests principles

Blood samples were analysed by Sysmex XE-5000 (Sysmex Corporation - Global - Kobe, Japan). Test principle in this device is based on the fluorescence flow cytometry method. Three parameters in FBC were considered; Haemoglobin (Hb), Haematocrit (Hct), Mean Corpuscular Volume (MCV). Concomitantly, samples for TSH, FT3, FT4 and Ferritin were assayed using The VITROS Immunodiagnostic System (VITROS ECI, Ortho-Clinical Diagnostics, Inc. Rochester NY, U.S.A.). All assays on the VITROS Analyzer employ an enhanced chemiluminescence detection reaction.

Serum iron level and transferrin were tested using an Architect C8000 (Abbott Laboratories. Abbott Park, Illinois, U.S.A.). Iron was determined by using the direct colorimetric method without deproteinisation in the human serum. Transferrin measurement on the other hand was based on an immunoturbidimetric procedure, which measures increasing sample turbidity caused by the formation of insoluble immune complexes.

Antenatal and cord blood serum aliquots for anti-TPO antibodies along with urine iodine samples were processed at the RPA, Sydney. Anti-TPO antibodies were measured using an IMMULITE 2000 systems analyzer (SEIMENS). It is a solid-phase, enzyme-labelled, chemiluminescent sequential immunometric assay.

Iodine in urine was measured directly by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) after a dilution in an ammonium EDTA based diluents in a quantitative application using rhodium as an internal standard. ICP-MS is an

analytical technique that is capable of performing quantitative multi-element analysis at trace concentrations of these elements. An ICP-MS is capable of measuring concentrations to a level of ng / L of the analyte.

2.4 Data collection, entry and storage

Data was collected using three sources of information. Firstly, questionnaire-derived information (the modified data capturing sheet) was used to express approximately one hundred parameters. A mixed of Qualitative and quantitative data was coded from this source. The second source was the LGH Pathology test results for FBC, IS, TSH, FT4 and FT3. Thirteen test parameters were obtained for each participant from this source. The third data source was the RPA, Sydney where results for urine iodine, urine creatinine and anti-TPO antibodies were collected. Data were entered on a daily basis into a password protected electronic data sheet and saved in the hospital O-drive. Original paper documents (consents, questionnaire and results) were sorted into numeric order according to participant's trial identification number, each in a separate folder and stored in locked drawers.

2.5 Data analysis

2.5.1 Methods of statistics

The rate of complication events in the mother and baby associated with various predictor variables was estimated as the incidence rate ratio (IRR; 95% confidence intervals; P-values) using multivariate Poisson regression. The covariates were selected using backward stepwise regression (P-value for removal = 0.22; P-value for entry = 0.12). An incidence rate ratio (IRR) of 1.00 indicates that the rate in the

group being compared with the comparator group (e.g. low thyroids with normal ones) is of no difference between the two groups; an IRR greater than 1.00 means the group has a higher rate than the comparator group; an IRR less than 1.00 means that the rate is lower than in the comparator group.

Variables for possible selection include for pre- and intra-natal complications: low thyroid status, BMI, first baby, history of prematurity or PET, gestation at delivery<37weeks, APH, hypertension/PET in this pregnancy, oligo- or poly-hydramnios, sex of baby, GDM, infection, placenta previa, TPL, IUGR, macrosomia, abdo/pelvic surgery, FBS, MCV, Hb. For post-natal complications the same list was used, plus prolonged labour, intra-partum haemorrhage and mode of delivery.

Where the complications were measured as a continuous variable (i.e. APGAR scores at 1minute and 5 minutes or head circumference), regression coefficients (coef; 95% confidence intervals; P-values) were estimated using multivariate general linear modelling: covariates were selected using backward stepwise regression. The covariates shown were each categorical variables, with the coefficient being the estimated difference found when the covariate is present. Mean and difference (95% confidence intervals; P-values) estimated by general linear modelling, adjusting for the same covariates for each complication.

Where continuous covariates were evaluated for association with outcomes, z-scores of variables were used ($\{variable\text{-}mean_{variable}\}/SD_{variable}$), using either natural value or log10 transformation. Z-scores have two advantages when used in multivariate regression analyses: 1) the regression constant is corrected to the mean of all of the

continuous variables (rather than to an abstract zero for all of the included variables, i.e. it remains in the middle of the data rather than at the edge of the raw data range); and 2) each variable is corrected to a single unit of value (units of 1 standard deviation), so that all variable coefficients have the same scale, and the magnitude of the association of each variable can be compared directly, rather than having to do complex mental arithmetic to correct for different units of measurement and value ranges.

In order to determine whether any symptoms or combination of symptoms elicited at the initial antenatal clinic visit could predict the presence of low thyroid status, the association between the results from the thyroid symptoms questionnaire and the subsequent determination of women with low thyroid status was estimated using multivariate Poisson regression, with variables for inclusion in the final model selected by backward stepwise regression. All the questions were included unless there was at least one positive result in both the low and normal thyroid status women (any zero results make interpretation of the regression coefficients extremely problematical). Pre-pregnancy tremor, weight loss, carpal tunnel syndrome and antenatal hair loss were excluded for this reason. The regression coefficients for the final model were then used to calculate for each woman a combined risk score (see Equation 1 in results section).

The optimum threshold for identification of low thyroid status was then selected, using receiver operating curve (ROC) analysis, based on judgement of the estimated sensitivity and specificity. The statistical significance of the estimated ROC area (whether the observations may have arisen by chance) was determined by

comparison of the ROC area with a normally distributed randomly-generated set of numbers with the same mean and standard deviation as the risk score. All analyses were performed using Stata MP2 versions 13.2 (StataCorp, College Station, Texas USA).

3 RESULTS

3.1 Description of the study population

During the study period, approximately a total of 2200 pregnant women of Northern Tasmania attended the pathology for gestational diabetes screening, mainly were referred from the Queen Victoria antenatal clinic, LGH. The target population were invited, 636 (28.9%) responded and consented to take part in the study. The study also includes data collected from 625 babies born to the mothers recruited to this study as well as investigation of their cord blood. Out of 636 participants, 609 (95.7%) were successfully screened for thyroid status. Gestational age at presentation and recruitment was around 26 weeks (median 26.4; inter-quartile range 25 to 27.6; range 12.6 to 37.7) with no difference in recruitment age distribution between the normal and low thyroid status groups ($P > 0.90$), (Figure 3-1).

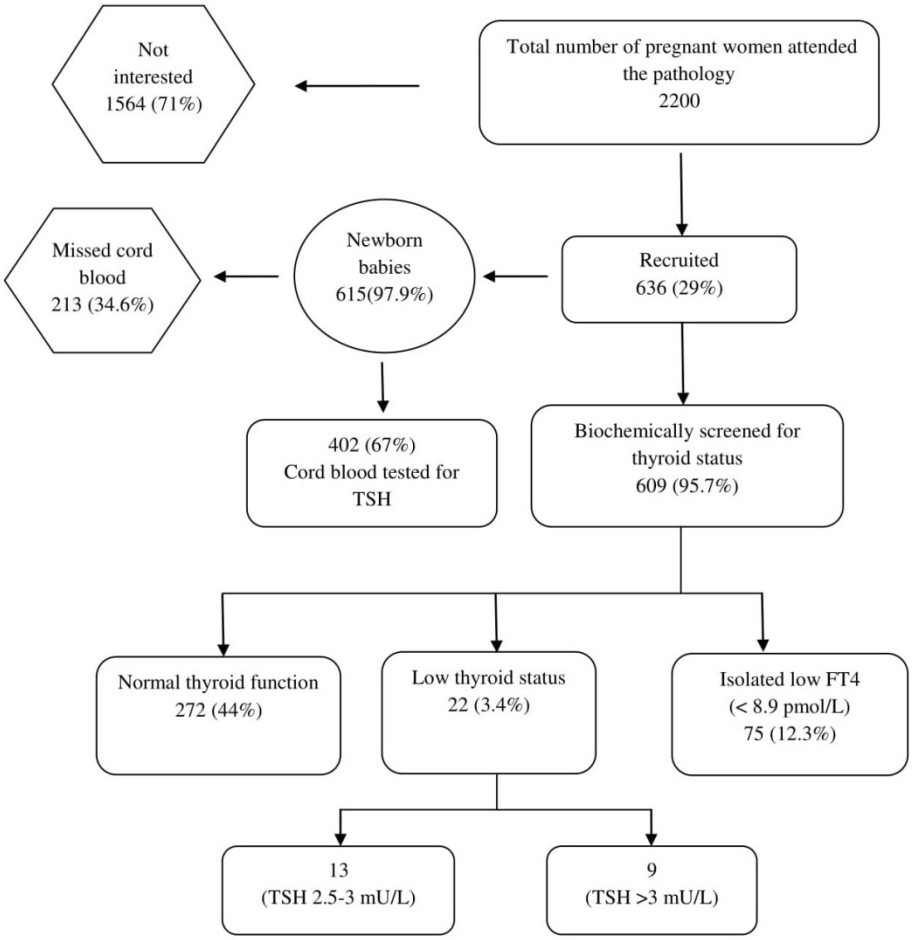

Figure 3-1 Study populations with response rate and recruitment

Response rate was 29% of 2200 pregnant women approached with invitation. Biochemical thyroid screening was processed to 95.7% of the participants and 67% of their babies cord blood. Low thyroid status was found in 3.4% of the pregnant cohort.

Using thyroid function testing markers (TSH, FT4 and FT3) as a screening tool, the study population was divided into three main categories; low thyroid status (TSH > 3mU/L and TSH >2.5mU/L), isolated low free thyroxin level (FT4 < 8.9pmol/L) and normal (comparator group). Among consented participants, 397 (63.1%) are multiparous (have previously been pregnant) and 232 (36.88%) are nulliparous (first time pregnant) with a median age of 29 years. The median age of gestation at recruitment was 26 weeks and gestational age at delivery was 39.4 weeks (Figure 3-2).

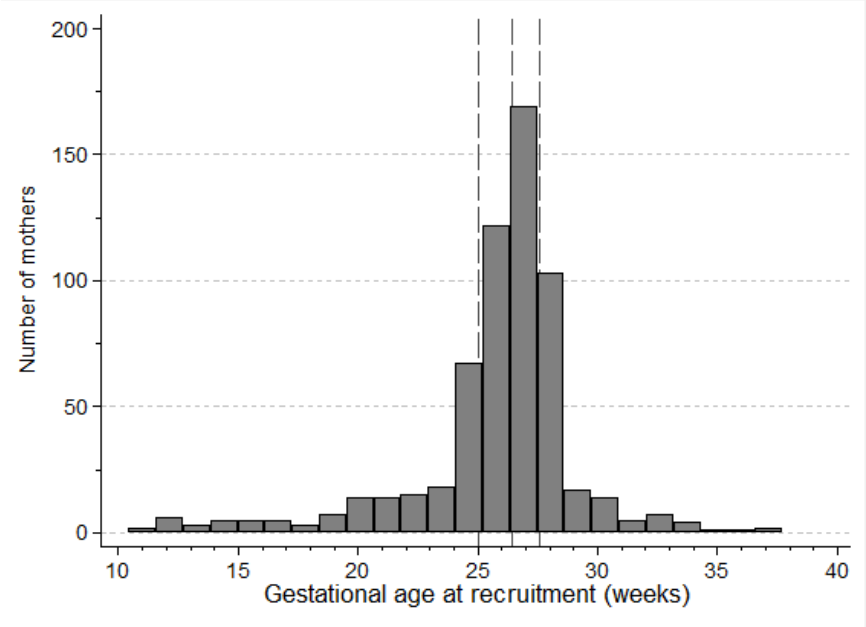

Figure 3-2 Distribution of gestational age in study population

Most participants were recruited when they were between 24 and 28 weeks of gestational age.

The study data also shows that 612 (96.2%) reported being conceived naturally. The breakdown of ethnic background was 503 (79%), 27 (4.24%), 11 (1.7%) and 4 (0.7%) representing Caucasian, Asian, indigenous Australian and African, respectively. No ethnic background was provided for 87 (13.7%) participants.

Data of sub-population of 625 babies' shows that 615 (97.9%) were born alive with male to female ratio of 1:1.04 (49% and 51% respectively). The total number of clinically observed complications for this study group is 269; 9 events (3.3%) are distributed in the group of low thyroid status, 135 (50.2%) events are distributed among the group category with low FT4 and 125 (46.5%) events of complications are distributed variably in the group of cohorts who have normal thyroid function.

3.2 Study outcomes

3.2.1 Incidence of thyroid abnormalities in the study population

Thyroid function screening results for its 609 pregnant women (Table 3.1) shows 22 (3.4%) with low thyroid function (9 with TSH > 3 mU/L and 13 with TSH 2.5-3 mU/L), 75 (12.3%) with free thyroxin level (FT4) < 8.9 pmol/L and 272 (44%) with results within the normal ranges for thyroid function. Table 3-1 shows the rate of all recorded complications in each of these groups.

Additionally, results of thyroid peroxidase (TPO) antibodies showed 39/585(6.3%) TPO-positive and 546/585 (93.3%) were negative. The average of TSH results was 1.2 mU/L in the TPO+ve group, not much different from 1.3 mU/L in the TPO-ve group.

Table 3-1 Ante-, intra- or post-natal complications in groups defined by thyroid status

	Total events	P-value[1]	Normal (N=272)		FT4 (8.9-15) (N=240)		FT4(<8.9) (N=75)		TSH[2](2.5-3.0) (N=13)		TSH[2](>3.0) (N=9)	
			n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Antepartum complications												
Oligohydroamnios	14	>0.90	7	(2.6%)	4	(1.7%)	3	(4.0%)	0	(0.0%)	0	(0.0%)
Polyhydromnios	5	>0.90	3	(1.1%)	0	(0.0%)	2	(2.7%)	0	(0.0%)	0	(0.0%)
GDM	49	0.70	23	(8.5%)	15	(6.3%)	9	(12.0%)	1	(7.7%)	1	(11.1%)
GHT/PET	38	0.39	22	(8.1%)	10	(4.2%)	6	(8.0%)	0	(0.0%)	0	(0.0%)
Infection	7	>0.90	3	(1.1%)	3	(1.3%)	1	(1.3%)	0	(0.0%)	0	(0.0%)
Placenta previa	5	0.17	1	(0.4%)	2	(0.8%)	1	(1.3%)	1	(7.7%)	0	(0.0%)
TPL	35	>0.90	18	(6.6%)	14	(5.8%)	2	(2.7%)	0	(0.0%)	1	(11.1%)
APH	9	>0.90	2	(0.7%)	7	(2.9%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
IUGR/organ malformation	16	>0.90	7	(2.6%)	8	(3.3%)	1	(1.3%)	0	(0.0%)	0	(0.0%)
Macrosomia	8	>0.90	2	(0.7%)	4	(1.7%)	2	(2.7%)	0	(0.0%)	0	(0.0%)
Biliary disease	6	0.20	4	(1.5%)	1	(0.4%)	0	(0.0%)	0	(0.0%)	1	(11.1%)
Surgery	4	>0.90	3	(1.1%)	1	(0.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Intrapartum complications												
Cord prolapse	0	0.036	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Prolonged labour	8		0	(0.0%)	5	(2.1%)	2	(2.7%)	0	(0.0%)	1	(11.1%)
Forceps	7	0.010	3	(1.1%)	4	(1.7%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
IPH	4	0.74	1	(0.4%)	1	(0.4%)	1	(1.3%)	1	(7.7%)	0	(0.0%)
Pyrexia	5	0.17	3	(1.1%)	0	(0.0%)	2	(2.7%)	0	(0.0%)	0	(0.0%)
HTN	9	>0.90	5	(1.8%)	3	(1.3%)	1	(1.3%)	0	(0.0%)	0	(0.0%)
Postpartum complications												
PPH	35	0.66	15	(5.5%)	13	(5.4%)	5	(6.7%)	1	(7.7%)	1	(11.1%)
Wound infection	5	0.62	3	(1.1%)	1	(0.4%)	1	(1.3%)	0	(0.0%)	0	(0.0%)

[1] P-value estimated using Fischer's exact test

[2] Estimate of proportion of cases in sample with elevated TSH results: 3.4% (95%CI 2.1% to 5.2%)

Abbreviations: **GDM** Gestational diabetes; **GHT/PET** Gestational hypertension/Pre-eclamptic toxaemia; **TPL** Threatened premature labour; **APH** Ante-partum haemorrhage; **IUGR** Intra-uterine growth retardation; **Surgery** Abdominal or pelvic surgery; **Forceps** Any instrumental delivery (e.g. forceps or Ventouse); **IPH** Intrapartum haemorrhage; **HTN** Hypertension; **PPH** Postpartum haemorrhage

3.2.2 Association of maternal and foetal complications rate with thyroid status

Table 3-1 shows the total number of all complications during three consecutive periods of pregnancy/labour and distribution rate of these complications among different thyroid status groups. Results in Table 3-2 summarise rates and associations of complications in the low thyroid status group. Because of the relatively low number in the result group (22 with low thyroid status), as well as the limited events of complications, it was difficult to clearly identify any small to moderate increase in their rate of incidence.

Results of the statistical analysis presented in tables 3-1 and 3-2 don't show any significant association between ante-natal complications and low thyroid status. Although GDM results in Table 3-2 affecting 2 out of 22 of the same group (IRR 1.69; 95% CI 0.55-5.25; P= 0.36) doesn't reflect a possible association with low thyroid status, covariates including BMI, log.fasting blood sugar and history of PET demonstrate significant relationship. While the former two show predictive values with (IRR 2.20; 95%CI 1.73 to 2.81; P < 0.001) and (IRR 1.25; 95%CI 1.00 to 1.55; P= 0.46), respectively, the latter shows protective values (IRR 0.63; 95%CI 0.41 to 0.96; P= 0.032).

Other associations between individual complications and different covariates are demonstrated in table 3-2; macrosomia is assumed to be affected more with other factors than with low thyroid status. Log.fasting blood sugar, BMI, maternal age, gravid>2 and gestational diabetes, show positive association with a great incidence increase in the last two with (IRR 13.4; 95%CI 2.34 to 76.6; P= 0.004) and (IRR 8.28; 95%CI 2.44 to 28.0; P= 0.001), respectively. While a positive association is

found between PET/Gestational hypertension and (log.haemoglobin, BMI and maternal age), history of PET, MCV and gravida >2 are negatively associated. Also, threatened premature labour is assumed predictable by increased history of prematurity (IRR 3.69; 95%CI 1.81 to 7.51; P < 0.001) and assumed protected by less history of PET (IRR 0.61; 95%CI 0.42 to 0.91; P= 0.014).

Although results couldn't clearly identify any significant association of GDM with low thyroid, it was positively associated with ante-partum haemorrhage (IRR13.9; 95%CI 4.79 to 213; P < 0.001). Table 3-3 reflects a possible association between prolonged labour (PL) and low thyroid status (IRR 3.31; 95% CI 1.13 to 9.69; P= 0.029). While there are no other intra-natal complications associated with low thyroid status, maternal age and log.ferritin were found positively associated with prolonged labour with 90% and 53% of incidence increase, respectively.

Table 3-2 Association of individual antenatal complications and low thyroid status

Gestational diabetes	Present	(%)	Absent	IRR	95%CI	P-value
Low Thyroid	2	(9.1%)	20	1.69	(0.55 to 5.25)	0.36
Normal	47	(8.0%)	540	1.00		
Covariates[2]						
Log. Fasting Blood Sugar[3]				2.20	(1.73 to 2.81)	<0.001
History of PET				0.63	(0.41 to 0.96)	0.032
BMI[3]				1.25	(1.00 to 1.55)	0.046
Macrosomia	Present	(%)	Absent	IRR	95%CI	P-value
Low Thyroid	0	(0.0%)	22	0.00	(0.00 to 0.00)	0.00
Normal	8	(1.4%)	579	1.00		
Covariates[2]						
MCV[3]				1.71	(0.94 to 3.10)	0.08
Log. Fasting Blood Sugar[3]				2.12	(1.30 to 3.47)	0.003
History of PET				4.73	(0.70 to 32.1)	0.11
BMI[3]				2.12	(1.40 to 3.21)	<0.001
Maternal age[3]				1.92	(1.14 to 3.22)	0.013
Gravida 1				1.00		
Gravida>2				13.4	(2.34 to 76.6)	0.004
Gestational diabetes				8.28	(2.44 to 28.0)	0.001
Infection	Present	(%)	Absent	IRR	95%CI	P-value
Low Thyroid	0	(0.0%)	22	0.00	(0.00 to 0.00)	<0.001
Normal	7	(1.2%)	580	1.00		
Covariates[2]						
History of Prematurity				3.42	(0.62 to 18.8)	0.16
Log. Ferritin[3]				1.40	(1.06 to 1.85)	0.017
IUGR/Organ malformation	Present	(%)	Absent	IRR	95%CI	P-value
Low Thyroid	0	(0.0%)	22	0.00	(0.00 to 0.00)	<0.001
Normal	16	(2.7%)	571	1.00		
Covariates[2]						

BMI[3]				0.69	(0.40 to 1.21)	0.19

PET / Gestational hypertension	Present	(%)	Absent	IRR	95%CI	P-value
Low Thyroid	0	(0.0%)	22	0.00	(0.00 to 0.00)	<0.001
Normal	38	(6.5%)	549	1.00		
Covariates[2]						
Log. Haemoglobin[3]				1.44	(1.10 to 1.88)	0.008
MCV[3]				0.70	(0.54 to 0.90)	0.005
Gravida 2				0.51	(0.26 to 1.00)	0.05
Gravida>2				0.30	(0.14 to 0.66)	0.003
History of Prematurity				1.90	(0.91 to 3.97)	0.09
History of PET				0.30	(0.17 to 0.52)	<0.001
BMI[3]				1.59	(1.24 to 2.03)	<0.001
Maternal age[3]				1.41	(1.07 to 1.85)	0.013
Threatened premature labour	Present	(%)	Absent	IRR	95%CI	P-value
Low Thyroid	1	(4.5%)	21	0.58	(0.14 to 2.44)	0.46
Normal	34	(5.8%)	553	1.00		
Covariates[2]						
MCV[3]				1.20	(0.90 to 1.60)	0.21
Log. Ferritin[3]				1.18	(0.93 to 1.49)	0.17
History of Prematurity				3.69	(1.81 to 7.51)	<0.001
History of PET				2.72	(0.68 to 10.9)	0.16
Maternal age[3]				0.61	(0.42 to 0.91)	0.014
Gravida 2				2.14	(0.96 to 4.77)	0.06
Gravida>2				1.88	(0.71 to 4.95)	0.20
Antepartum haemorrhage	Present	(%)	Absent	IRR	95%CI	P-value
Low Thyroid	0	(0.0%)	22	0.00	(0.00 to 0.00)	<0.001
Normal	9	(1.5%)	578	1.00		
Covariates[2]						
Log. Fasting Blood Sugar[3]				0.45	(0.23 to 0.87)	0.02
History of Prematurity				Large	(Large to Large)	<0.001
BMI[3]				0.31	(0.11 to 0.89)	0.03

Maternal age[3]	1.64	(0.99 to 2.71)	0.05
Gravida 2	10.7	(0.84 to 135)	0.07
Gravida>2	5.15	(0.57 to 46.8)	0.15
Gestational diabetes	31.9	(4.79 to 213)	<0.001

1 Low thyroid status defined as TSH>2.5

2 Incidence rate ratio (IRR; 95% confidence intervals; P-values) estimated using multivariate Poisson regression: covariates were selected using backward stepwise regression, retained when P<0.22)

3 Continuous covariates used z-scores of variables ({variable-$mean_{variable}$}/$SD_{variable}$), using either natural value or log10 transformation

Table 3-3 Association of individual intra-natal complications and low thyroid status[1]

Prolonged labour	Present	(%)	Absent	IRR[2]	95%CI	P-value
Low Thyroid	1	(4.8%)	21	3.31	(1.13 to 9.69)	0.029
Normal	7	(1.2%)	580	1.00		
Covariates[2]						
Log. Ferritin[3]				1.53	(1.08 to 2.19)	0.018
Maternal age[3]				1.90	(1.04 to 3.47)	0.036

1 Low thyroid status defined as TSH>2.5

2 Incidence rate ratio (IRR; 95% confidence intervals; P-values) estimated using multivariate Poisson regression: covariates were selected using backward stepwise regression, retained when P<0.22)

3 Continuous covariates used z-scores of variables ({variable-$mean_{variable}$}/$SD_{variable}$) , using either natural value or log10 transformation

Despite the fact that symptoms of thyroid disease are non-specific, the predictive association between possible symptoms of thyroid disease at clinic visit and the discovery of low thyroid function biochemically was also investigated, Table 3-4. Palpitation (pre-pregnancy) was positively associated with low thyroid status as well as with normal thyroid function; 5 (22.7%), 73 (12.6%), respectively (IRR 2.78; 95% CI 1.00 to 7.70; P= 0.05). Dizziness (pre-pregnancy) also shows association with low thyroid group 10 (45%) and 189 (32%) in the normal group (IRR 2.15; 95% CI 0.88 to 5.23; P= 0.09). there were no other statistically significant associations.

A combined risk score for low thyroid status was created from the coefficients of this analysis using Equation 1 (the coefficient values are the natural logs of the IRRs from Table 3-4):

$$Risk\ score = -2.8676 + (PaplsPP * 1.0215) + (DizzyPP * 0.7647) + (DyspNow * -0.492) + (IrritableNow * -1.3638) + (DiarrhoeaNow * -0.7612)$$

This risk score had a range of values as shown in Figure 3-3. A threshold for prediction of the presence of low thyroid status was investigated using receiver operating characteristic (ROC) analysis, which suggested an optimum threshold value of -3.25 and above for this risk score. Comparison of the ROC area with the random null hypothesis showed a ROC area of 0.744 (95%CI 0.636 to 0.852; Chi^2=8.05; P=0.005). Using this value (-3.25), 15 of 22 (sensitivity or true positive 68.2%; 95%CI 45.1% to 86.1%) patients with low thyroid status were identified as possible cases whilst 164 of 580 (1-specificity or false positive 28.3%; 95%CI 24.6% to 32.1%) patients with normal thyroid status were identified as possible cases (IRR 5.15; 95%CI 2.13 to 12.4; P < 0.001).

However, relatively large numbers of patients with low and normal thyroid status had risk scores close to the threshold of -3.25, and minor changes to the threshold chosen appeared to produce large alterations in the sensitivity and specificity estimates for the risk score.

Table 3-4 The predictive association between symptoms of possible thyroid disease at clinic visit and the discovery of low thyroid function[1]

Variable	Low thyroid N (% of 22)	Normal thyroid N (% of 580)	IRR[2]	95%CI	P-value
Variables included in prediction model					
Palpitations PP	5 (22.7%)	73 (12.6%)	2.78	(1.00 to 7.70)	0.05
Dizzy PP	10 (45.5%)	189 (32.6%)	2.15	(0.88 to 5.23)	0.09
Irritable Now	3 (13.6%)	183 (31.6%)	0.26	(0.08 to 0.84)	0.024
Dyspnoea Now	9 (40.9%)	328 (56.6%)	0.59	(0.26 to 1.31)	0.19
Diarrhoea Now	9 (40.9%)	348 (60.0%)	0.47	(0.21 to 1.05)	0.06
Variables not included in model: Each IRR is variable effect when combined with the above model					
Tired PP	6 (27.3%)	125 (21.6%)	1.42	(0.49 to 4.16)	0.52
Tired Now	13 (59.1%)	404 (69.7%)	0.98	(0.42 to 2.27)	>0.90
Constipation PP	2 (9.1%)	31 (5.3%)	1.26	(0.33 to 4.75)	0.74
Constipation Now	8 (36.4%)	194 (33.4%)	1.00	(0.44 to 2.26)	0.99
Heat intolerance PP	3 (13.6%)	72 (12.4%)	1.22	(0.36 to 4.14)	0.75
Heat intolerance Now	11 (50.0%)	256 (44.1%)	1.35	(0.62 to 2.93)	0.45
Cold intolerance PP	8 (36.4%)	197 (34.0%)	1.05	(0.46 to 2.41)	0.90
Cold intolerance Now	3 (13.6%)	135 (23.3%)	0.56	(0.17 to 1.87)	0.35
Insomnia PP	4 (18.2%)	135 (23.3%)	0.75	(0.25 to 2.25)	0.60
Insomnia Now	8 (36.4%)	221 (38.1%)	0.99	(0.40 to 2.47)	>0.90
Hair loss PP	1 (4.5%)	68 (11.7%)	0.31	(0.04 to 2.48)	0.27
Leg cramps PP	3 (13.6%)	80 (13.8%)	0.83	(0.23 to 2.97)	0.78
Leg cramps Now	12 (54.5%)	295 (50.9%)	1.32	(0.55 to 3.16)	0.54
Carpal tunnel syndrome Now	3 (13.6%)	80 (13.8%)	1.21	(0.33 to 4.37)	0.77
Palpitations Now	6 (27.3%)	172 (29.7%)	0.67	(0.29 to 1.56)	0.35

Arrhythmias PP	2 (9.1%)	46 (7.9%)	0.89	(0.21 to 3.77)	0.88
Arrhythmias Now	2 (9.1%)	53 (9.1%)	0.93	(0.24 to 3.70)	0.92
Dyspnoea PP	2 (9.1%)	55 (9.5%)	0.89	(0.17 to 4.66)	0.89
Irritable PP[3]	5 (22.7%)	135 (23.3%)	2.47	(0.94 to 6.50)	0.07
Nausea & vomiting PP	2 (9.1%)	16 (2.8%)	1.93	(0.47 to 8.01)	0.36
Nausea & vomiting Now	10 (45.5%)	273 (47.1%)	1.11	(0.48 to 2.52)	0.81
Diarrhoea PP	13 (59.1%)	400 (69.0%)	1.58	(0.55 to 4.48)	0.39
Weight gain PP	1 (4.5%)	54 (9.3%)	0.59	(0.08 to 4.19)	0.60
Weight gain Now	2 (9.1%)	44 (7.6%)	1.32	(0.34 to 5.16)	0.69
Weight loss Now	1 (4.5%)	22 (3.8%)	0.82	(0.08 to 8.37)	0.87
Depression PP	7 (31.8%)	211 (36.4%)	0.97	(0.38 to 2.49)	>0.90
Depression Now	3 (13.6%)	80 (13.8%)	1.44	(0.41 to 5.12)	0.57
Tremor Now	1 (4.5%)	38 (6.6%)	0.62	(0.08 to 4.83)	0.65
Headache PP	13 (59.1%)	272 (46.9%)	1.61	(0.67 to 3.88)	0.29
Headache Now	10 (45.5%)	313 (54.0%)	0.86	(0.37 to 2.03)	0.73
Dizzy Now	12 (54.5%)	310 (53.4%)	1.00	(0.37 to 2.66)	>0.90

[1] Low thyroid function defined as TSH<2.5.

[2] Association between predictive variables and low thyroid function, expressed as incidence rate ratio (IRR; 95% confidence intervals; P-values), estimated by Poisson regression; variables to be included in the final model were selected by backward stepwise regression from the list of variables shown in the table (PP=symptoms present pre-pregnancy; Now= symptoms present at the clinic visit).

[3] The variable Irritable PP was excluded from the final model: the prevalence in the two groups was similar, and the apparent possible association (IRR 2.47; P=0.07) was judged to be a statistical quirk. Inclusion of the variable in ROC analysis made little difference to the final results. Abbreviations: PP (pre-pregnancy), Now (during pregnancy)

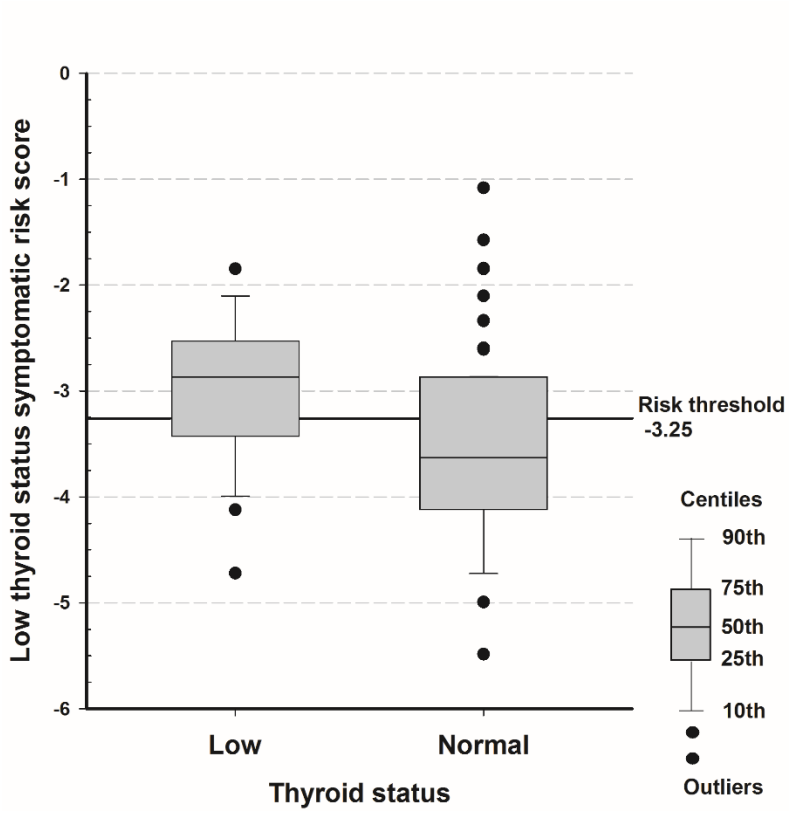

Figure 3-3 Distribution of predictive risk score in patients with low or normal thyroid status

Relatively large numbers of patients with low and normal thyroid status had risk scores close to the threshold of -3.25, and minor changes to the threshold chosen appeared to produce large alterations in the sensitivity and specificity estimates for the risk score.

3.2.3 Association of rates of neonatal complications with thyroid status

Apgar scores, head circumference, resuscitation and nursery admission were the parameters defining outcomes of neonates born to mothers with different thyroid status. Mean Apgar scores at 1 minute of 8.69 (SD 1.08; 95% CI -0.11 to 0.82; P= 0.14) and at 5 minutes value of 9.39 (SD 1.08; 95% CI -0.22 to 0.43; P= 0.52) in the low thyroid group are similar to the normal group (Table 3-5). Similarly, newborns' head circumference in table 3-6 show results with a mean value of 32.3 (SD 1.41; 95% CI -0.60 to 0.58; P > 0.90) among babies born to mothers with low thyroid, as well as the normal ones. These values do not demonstrate any association between babies head circumference and the thyroid status of the mothers. Likewise, the requirement for resuscitation and nursery admission to the babies (Tables 3-7, and 3-8) do not show an association with their mothers' thyroid condition.

Apart from the statistically insignificant association between the requirement of resuscitations and low thyroid status, other predictors show positive association (table 3-8) including delivery < 37 weeks, IUGR, IPH and placenta previa. Nursery admission also was associated positively with other predictors such as delivery < 37 weeks, GHT/PET, GDM, infection, IUGR and LSCS.

Table 3-5 Apgar scores at 1 and 5 minutes born to mothers with low or normal thyroid status

	n<8	(%)	n 8+	Mean Apgar	(SD)	Coef.[2]	95%CI	P-value
Apgar at 1 minute								
Low Thyroid[1]	3	(13.6%)	19	8.69	(1.08)	0.35	(-0.11 to 0.82)	0.14
Normal	98	(16.7%)	489	8.34	(2.23)			
Covariates[2]								
Delivery<37weeks						-0.56	(-1.11 to 0.00)	0.050
Male baby						-0.21	(-0.49 to 0.07)	0.14
IPH						-1.21	(-2.17 to -0.25)	0.014
Apgar at 5 minutes								
Low Thyroid[1]	1	(4.5%)	21	9.39	(1.08)	0.11	(-0.22 to 0.43)	0.52
Normal	31	(5.3%)	556	9.28	(2.23)			
Covariates								
Delivery<37weeks						-0.66	(-1.14 to -0.18)	0.007
Male baby						-0.25	(-0.47 to -0.04)	0.019
IPH						-0.37	(-0.95 to 0.21)	0.21
LSCS						0.24	(0.03 to 0.44)	0.023

1 Low thyroid status defined as TSH>2.5; numbers shown are those with Apgar scores < 8 or 8-10

2 Regression coefficient (the estimated difference found when the covariate is present)(coef.; 95% confidence intervals; P-values) estimated using multivariate general linear modelling: covariates were selected (low thyroid status, BMI, first baby, history of prematurity or PET, gestation at delivery<37weeks, APH, hypertension/PET in this pregnancy, oligo- or poly-hydramnios, sex of baby, GDM, infection, placenta previa, TPL, IUGR, macrosomia, abdo/pelvic surgery, FBS, MCV, Hb) using backward stepwise regression, retained when P<0.22); the covariates shown were each categorical variables

Table 3-6 Head circumference of babies born to low thyroid status mothers or normal mothers

	Mean	(SD)	Coef.[2]	95%CI	P-value
Low Thyroid[1]	32.3	(1.41)	-0.01	(-0.60 to 0.58)	>0.90
Normal	32.3	(1.69)			
Covariates					
BMI[3]			0.28	(0.15 to 0.40)	<0.001
APH			-1.68	(-2.54 to -0.81)	<0.001
Oligohydroamnios			0.72	(-0.11 to 1.55)	0.09
Male baby			0.34	(0.10 to 0.58)	0.006
GDM			-0.47	(-0.98 to 0.04)	0.07
Infection			-1.08	(-1.93 to -0.23)	0.013
Macrosomia			1.89	(0.91 to 2.86)	<0.001
Abdo/pelvic surgery			-1.89	(-3.20 to -0.58)	0.005
Delivery<37weeks			2.24	(1.59 to 2.90)	<0.001

1 Low thyroid status defined as TSH>2.5; numbers shown are those with Apgar scores < 8 or 8-10

2 Regression coefficient (coef.; 95% confidence intervals; P-values) estimated using multivariate general linear modelling: covariates were selected (low thyroid status, BMI, first baby, history of prematurity or PET, gestation at delivery<37weeks, APH, hypertension/PET in this pregnancy, oligo- or poly-hydramnios, sex of baby, GDM, infection, placenta previa, TPL, IUGR, macrosomia, abdo/pelvic surgery, FBS, MCV, Hb) using backward stepwise regression, retained when P<0.22); the covariates shown were each categorical variables, with the coefficient being the estimated difference found when the covariate is present

3 Continuous covariates used z-scores of variables ({variable-$\text{mean}_{\text{variable}}$}/$\text{SD}_{\text{variable}}$), using either natural value or log10 transformation

Abbreviations: **APH** Antepartum haemorrhage; **GDM** Gestational diabetes

Table 3-7 Requirement for resuscitation of babies born to low thyroid status mothers or normal mothers

	Required	(%)	None	IRR[2]	95%CI	P-value
Low Thyroid[1]	3	(13.6%)	19	0.78	(0.31 to 1.94)	0.59
Normal	95	(16.2%)	492	1.00		
Covariates						
BMI[3]				1.13	(0.95 to 1.35)	0.16
Placenta Previa				6.26	(3.38 to 11.60)	<0.001
GHT/PET				1.69	(0.99 to 2.88)	0.05
TPL				1.50	(0.93 to 2.43)	0.10
IUGR				2.36	(1.17 to 4.73)	0.016
Prolonged labour				2.08	(0.67 to 6.43)	0.21
IPH				2.32	(1.50 to 3.60)	<0.001
Gravida 1				1.44	(1.00 to 2.07)	0.05
Delivery<37weeks				2.69	(1.81 to 3.98)	<0.001

1 Low thyroid status defined as TSH>2.5

2 Incidence rate ratio (IRR; 95% confidence intervals; P-values) estimated using multivariate Poisson regression: covariates were selected using backward stepwise regression, retained when P<0.22)

3 Continuous covariates used z-scores of variables ({variable-$\text{mean}_{\text{variable}}$}/$\text{SD}_{\text{variable}}$), using either natural value or log10 transformation
Abbreviations: **GHT/PET** Gestational hypertension/Pre-eclamptic toxaemia; **TPL** Threatened premature labour; **IUGR** Intra-uterine growth retardation; **IPH** Intrapartum haemorrhage

Table 3-8 Requirement for nursery admission of babies born to low thyroid status mothers or normal mothers

	Nursery	(%)	None	IRR[2]	95%CI	P-value
Low Thyroid[1]	1	(4.5%)	21	0.38	(0.12 to 1.24)	0.11
Normal	58	(9.9%)	529	1.00		
Covariates						
BMI[3]				1.04	(0.78 to 1.38)	0.81
History of Prematurity				0.94	(0.54 to 1.65)	0.83
History of PET				1.99	(0.90 to 4.36)	0.09
GHT/PET				3.71	(1.78 to 7.71)	<0.001
GDM				2.00	(1.18 to 3.38)	0.010
Infection				3.50	(1.14 to 10.8)	0.029
Placenta previa				3.24	(1.56 to 6.73)	0.002
TPL				1.84	(1.04 to 3.25)	0.035
IUGR/organ				3.69	(1.87 to 7.25)	<0.001
Surgery				3.23	(1.16 to 9.04)	0.025
MCV[3]				1.74	(1.31 to 2.31)	<0.001
Delivery<37weeks				6.51	(4.08 to 10.4)	<0.001
LSCS				2.04	(1.31 to 3.18)	0.002

1 Low thyroid status defined as TSH>2.5

2 Incidence rate ratio (IRR; 95% confidence intervals; P-values) estimated using multivariate Poisson regression: covariates were selected using backward stepwise regression, retained when P<0.22)

3 Continuous covariates used z-scores of variables ({variable-$mean_{variable}$}/$SD_{variable}$), using either natural value or log10 transformation
Abbreviations: **GHT/PET** Gestational hypertension/Pre-eclamptic toxaemia; **GDM** Gestational diabetes; **TPL** Threatened premature labour; **IUGR** Intra-uterine growth retardation; **IPH** Intrapartum haemorrhage; **LSCS** Lower segment Caesarean section

3.3 Other considerations

3.3.1 Association between low thyroid status and iron deficiency anaemia

Ferritn level (body iron reserve) in the study cohort was used to define Iron Deficiency Anaemia. Results show that 193 pregnant women out of 590 (32.7%) have iron store level below 10mg/l. Although these results do not demonstrate association between ferritin level and thyroid status, data in (Table 3-9) clearly define the presence of macrocytic anaemia in 5 out of 22 participants whose TSH screening revealed low thyroid status.

3.3.2 Examination of surveillance of thyroid function using urinary iodine

Urinary Iodine is another proposed predictor in this study. We were able to successfully screen 587 participants, all under similar conditions (time and place). Calculation of the median urinary iodine concentration resulted in 117 µg/L. However, an adequate iodine intake for pregnant women is best achieved when median UIC is between 150-249 µg/L levels according to the latest recommendation by WHO (World Health Organization, 2013). Thus, the result of 117µg/L still reflects inadequate iodine intake for our pregnant women. Furthermore, 27/241 (11.2%) iodine deficient participants developed isolated hypothyroxinemia during pregnancy. Additionally, results of median UIC was correlated with results of urine creatinine of the same cohort. Although r (592) =0.44 reflects almost a large positive effect, relevant p-value of (p=3.1) however indicates statistically a non-significant correlation.

In order to find a possible association between (UIC and TSH) and (UIC and FT4), correlation and regression formulas were used. Data analysis revealed very little effect with no statistical significance r (588) = 0.017, p=0.67 and r (588) = 0.022, p=0.55 for (UIC and TSH) and (UIC and FT4), respectively.

3.3.3 Study results-based reference group

TSH results (irrespective to TPO status) in the study group have been re-calculated, they fall between the range of 0.16 mU/L (95% CI 0.75, 0.64) and 2.64 mU/L (95% CI 1.93, 3.34).

Similarly, results for FT4 of the study cohort have also been re-calculated for the group reference range, LL =7.10 pmol/L (95% CI 5.36, 8.83) and UL =14.02pmol/L (95% CI 12.28, 15.75).

Data derived from TPOAB results in this study has however been calculated. Out of 585 of total participants, 546 (93.3%) showed negative results for TPO antibodies. Nevertheless, their TSH results range between 0.15mU/L (95% CI 0.84, 0.53) and 2.6mU/L (95% CI 1.9, 3.3).

TPO antibodies were tested positive only in 39/585 participants (6.6%). However, their TSH results are distributed between 0.04mU/L (95% CI 0.8, 0.76) and 3.05mU/L (95% CI 2.27, 3.82).

Calculation of cutoff values for TSH according to TPO status revealed almost 0.5mU/L difference in favor of TPO positive group where only two of TSH results showed above 3.05mU/L and similar number of participants has UIC < 117ug/L without any identifiable association between them. However, because of the small number of positive TPO results, we cannot draw a valid conclusion without making unsubstantiated assumptions.

Table 3-9 Association of low thyroid status[1] with various forms of anaemia, including microcytic (iron-deficiency) anaemia

Group[2]	N	Group total	% Group[3]	% Total[3]	Mean ferritin (SD)	IRR[4]	95%CI	P-value[5]
No anaemia	9	256	3.5%	1.5%	18.8 (18.7)	1.00		
Normocytic anaemia	5	175	2.9%	0.8%	32.2 (105.0)	0.81	(0.28 to 2.39)	>0.90
Microcytic anaemia	4	134	3.0%	0.7%	8.3 (5.5)	0.85	(0.27 to 2.71)	0.78
Macrocytic anaemia	4	25	16.0%	0.7%	31.1 (43.5)	4.55	(1.51 to 13.74)	0.022

1 Low thyroid status defined as TSH>2.5.

2 Women were classified as having: 1) No anaemia (Hb≥120); 2) Normocytic anaemia (Hb<120, MCV 85-96); 3) Microcytic anaemia (Hb<120, MCV <85 or Ferritin <10); 4) Macrocytic anaemia (Hb<120, MCV >96).

3 Percentages were calculated as % of each group, and % of total (N=590) women.

4 Likelihood of occurrence of low thyroid status was estimated as incidence rate ratio (IRR; 95% confidence intervals; P-values) using Poisson regression with no covariates.

5 P-values were adjusted for multiple comparisons using the Holm method.

3.3.4 Cord blood TSH

Cord blood samples of 402/615 (65%) babies were collected and the reasons for low percentage in cord blood collection were; either due to insufficient samples or simply forgotten during labour chaos. Cord TSH results in table 3.10 (Mean 8.11; 95% CI -2.74 to 0.4; P= 0.008) of 121 babies born by operative lower segment Caesareans section (LSCS), demonstrate relatively lower TSH levels compared to results of babies born by instrumental support or those who were born normally.

Table 3-10 reflects results of babies born to participants with bleeding complications (APH/IPH), show lower estimates compared to participants without bleeding (Mean (SD) 4.91(2.16); 95% CI -5.48 to -2.48; P < 0.001). Similarly, the same table shows that cord TSH results of babies born to women with previous history of pregnancy are slightly lower compared to those who were born to first time pregnant participants.

Table 3-10 Association between predictor variables and levels of cord TSH[1]

	N	Mean (SD) [2]	Mean Δ[2]	95%CI	P-value
Normal thyroid	387	9.27 (5.92)	0.00		
Low thyroid	15	9.81 (6.66)	0.45	(-2.62 to 3.53)	0.77
Normal vaginal delivery	222	9.73 (6.22)	0.00		
Instrumental delivery	59	10.07 (6.89)	-0.38	(-2.35 to 1.58)	0.70
LSCS[4]	121	8.11 (4.67)	-1.57	(-2.74 to -0.41)	0.008
No history	367	9.21 (5.98)	0.00		
History of prematurity	35	10.17 (5.60)	1.53	(-0.39 to 3.45)	0.12
No haemorrhage	392	9.40 (5.97)	0.00		
APH or IPH	10	4.91 (2.16)	-3.98	(-5.48 to -2.48)	<0.001
Normal labour	396	9.33 (5.96)	0.00		
Prolonged labour	6	6.74 (3.75)	-2.09	(-5.14 to 0.96)	0.18
Primigravida	145	10.43 (6.72)	0.00		
Multigravida	257	8.65 (5.36)	-1.92	(-3.23 to -0.62)	0.004
Log10 Hb (reg. coef[3])	402	2.07 (0.03)	0.53	(0.08 to 0.99)	0.022

1 Cord TSH measurements were collected in 402 patients

2 The mean (standard deviation) and mean difference between presence or absence of predictor variables was estimated using general linear modelling (95% confidence intervals; P-values). Predictor variables to be included in the model were selected using backwards stepwise regression (P-value for removal = 0.22; P-value for entry = 0.12), with low thyroid status as a forced entry variable. For the categorical variables, the base comparator variable is assigned a mean difference of 0.

3 For haemoglobin, log10 transformation was used due to non-normal distribution, and this value was further transformed as a z-score ((Hb-mean)/SD): the regression coefficient (95% confidence intervals; P-value) shown is the rise in cord TSH associated with each 1 standard deviation rise in Log10 Hb.

4 No difference in the mean difference of cord TSH was seen between emergency (N=43) and elective (N=78) lower segment Caesarean section (0.01; 95%CI 2.03 to -2.02; P>0.90)

4 DISCUSSION AND CONCLUSION

This study has demonstrated that approximately 3.4% of the Northern Tasmanian women could be at risk of developing thyroid dysfunction during their pregnancy with (TSH>2.5-3mU/L and >3mU/L) when measured between end of the second trimester and start of the third. Additionally, 12.3% of the same pregnant population could be labelled with the controversial status of (Isolated Hypothyroxinemia) when applying <8.9pmol/L for FT4 as a cut-off. The results also suggest that antenatal complications defined in this study are not associated with low thyroid status. The outcomes of this study also suggest that the risk for experiencing prolonged labour as an intra-natal complication can be increased up to three-fold with low thyroid status (IRR 3.31; 95% CI 1.13 to 9.69; P= 0.029).

There was no positive association between iron deficiency anaemia (IDA) and low thyroid status could be demonstrated in this thesis. This study was not able to clearly demonstrate whether a symptom based risk score for thyroid dysfunction during pregnancy would be better than biochemical screening. Although CB-TSH demonstrated a positive association with maternal complications (IPH), its role in assessing foetal thyroid status has not been established. A 41% of the study population had iodine deficiency during pregnancy and 11.2% of this subpopulation developed isolated hypothyroxinemia.

Despite more than 2200 subjects being approached to enrol in the study, the consent rate was relatively low (28%). The main reason for this is most likely to be the single-centred study design, as well as the general lack of awareness of the consequences of iodine deficiency within the targeted study population. Although it was expected that the incidence rate of low thyroid function during pregnancy would

be higher than 3.4%, this is in line with internationally estimated figures for subclinical hypothyroidism in pregnancy (1.5-4 %), (Allan et al., 2000, Casey et al., 2005, Negro and Mestman, 2011). Furthermore, the results of this study suggest that the rate of prolonged labour as an intra-natal complication was greater in pregnant women with decreased thyroid function (IRR 3.31; 95% CI 1.13 to 9.69; P= 0.029).

There has been conflicting reports on the association between gestational diabetes and hypothyroidism (Casey et al., 2007, Cleary-Goldman et al., 2008, Korevaar et al., 2013). No clear association (P= 0.36) between these were shown in the current study. It has previously been suggested that finding associations between complications occurring during pregnancy and thyroid function disorder was a difficult task (Negro and Mestman, 2011). Furthermore, it has been reported that inappropriate sample size is a common problem which occurs while investigating complication rates in overt or subclinical hypothyroidism during pregnancy (Negro and Mestman, 2011). These are in agreement with the current study.

Although epidemic goitre is a strong part of Tasmanian history, the main culprit (iodine deficiency) remains persistent (Gibson, 2006, Burgess et al., 2007). It has also been reported that iodine deficiency is the main reason for subclinical hypothyroidism (SH) in iodine deplete areas (de Benoist et al., 2003, Hynes et al., 2004, Li et al., 2006, Andersson et al., 2007, Vanderpump, 2010). Despite iodine-fortification in Tasmania being mandatory, a nine year follow-up study of school-aged children who were born to iodine deficient mothers were found to have difficulties in reading and grammar when compared to peers of the same age (Burgess et al., 2007, Hynes et al., 2013).

The symptom-based risk score using commonly accepted indicators of thyroid dysfunction has some ability to differentiate low and normal thyroid status. There doesn't appear to be clear rationale for the variables included in the risk score. It may require orders of magnitude larger patient groups to reliably identify all the possible symptomatic expressions of low thyroid status. Also, the relatively small numbers of patients found with low thyroid status, as well as the relatively unstable estimates associated with nearby alternative thresholds, means that uncertainties around the usefulness of the risk score need to be considered.

The estimation of the coefficients to be included in the risk score, and the calculation of the patient risk scores for the purpose of evaluation of efficacy of differentiation were performed on the same patients, rather than on two separate groups, as would be preferable (due to insufficient numbers of patients with low thyroid status). A broader objection to this particular expression of a symptomatic screening test for potential low thyroid status is that it was not conducted completely in a primary antenatal clinic where pregnant women undergo a routine check-up from early gestational age with consequent follow-up throughout all pregnancy period.

IDA during pregnancy has been previously reported to cause obstetric and neonatal complications as well as neuro-psychological development (Scholl and Hediger, 1994, Brabin et al., 2001, Sachdev et al., 2005). A positive relationship between IDA and low thyroid function has also been shown (Zimmermann et al., 2000). Although the current study demonstrates low ferritin level in 32.7% of study participants, there is no evidence of associations with low thyroid status.

In contrast, macrocytic anaemia was reported in 4 of 22 (18%) of low thyroid status. In another study, 57.1% of participants with hypothyroidism were reported to have either microcytic or macrocytic anaemia (Omar et al., 2010), with similar findings different explanations reported by (Tudhope and Wilson, 1960, Fein and Rivlin, 1975, Horton et al., 1976). Although causes of macrocytosis in the general population can vary, including drugs, alcoholism, hypothyroidism and bone marrow dysplasia, deficiency of vitamin-B_{12} and folate were most prevalent in the obstetric population (Van der Weyden and Campbell, 1988, Colon-Otero et al., 1992, Savage et al., 2000, Kaferle and Strzoda, 2009, Tripathi et al., 2012). Further studies are required to differentiate that macrocytosis found in this study subgroup of low thyroid status, against those caused by vitamin-B_{12} and folate deficiencies.

The effect of iodine deficiency on the incidence of thyroid dysfunction has also been investigated. Although iodised salt has been promoted for the last decade and consumption of iodine-contained multi-vitamins, median urinary iodine of 117µg/L derived from study cohort results reflects deficiency according to WHO's latest recommendations (World Health Organization, 2013). While 11.2% of the study iodine deficient population was labelled with isolated hypothyroxinemia (IH), it was reported to be approximately of 25-30% in other iodine deficient population (Berbel et al., 2009, Moleti et al., 2009, Krassas et al., 2010).

Normal reference range for FT4 in the general population provided by LGH pathology is 10.00-28.2pmol/L. Thus, considering the fact of physiological changes in the thyroid regulation that occur in pregnancy (discussed earlier), a lower cutoff was considered. Since there is no standard reference range to define

hypothyroxinemia, lower bound (<8.9pmol/L) of FR4 used in the study was a result of statistical assumptions. Nevertheless, now we can set our own reference range for FT4 based on study cohort results. Results of free thyroxin of the study cohort have been recalculated for the group reference range, LL =7.10pmol/L (95% CI 5.36, 8.83) and UL =14.02pmol/L (95% CI 12.28, 15.75).

Applying the group lower bound (<7.1) as a cutoff will result in a remarkable decrease in number of isolated hypothyroxinemia to only 1.47% (9/609). Further clinical studies are required to justify the validity these biomedical references. The relevance of iodine deficiency and IH with possible health consequences on foetal neuropsychological development were also investigated and published (Morreale de Escobar et al., 2000, Negro and Mestman, 2011). Although a recent publication reported a negative effect of maternal iodine deficiency on offspring's reading ability, it was not verified whether IH was a contributing factor (Hynes et al., 2013). It is therefore recommended to follow-up of the iodine deficient population of this study, to determine whether neuropsychological consequences are due to iodine deficiency alone, IH alone or when combined.

It has been suggested that maternal IH has no impact on normal IQ development of offspring, according to a recent study (Grau et al., 2015). In contrast, association between hypothyroxinemia and autism has been suggested (Román et al., 2013). Also, a survey review of the European clinical institutions resulted in lack of a standard approach regarding hypothyroxinemia in pregnancy (Vaidya et al., 2012). These reports encourage further follow-up of IH cases of this study to be considered.

Persistence of iodine deficiency in Tasmania was reported a decade ago. In view of this fact, it was hoped that mandatory fortification and awareness would halt this public health issue. Nevertheless, median UIC of 117ug/L of our pregnant participants still reflects inadequate iodine intake. According to the latest recommendation by WHO; an adequate iodine intake for pregnant women is best achieved when median UIC is between 150-249 µg/L levels (World Health Organization, 2013). Retrospective analysis of this population and data related to their diet maybe required in order to investigate the causes and factors contributed to this result. Also, comparing this outcome with a non-pregnant population will lead to determine individual factors from combined ones.

Neonatal hypothyroidism causes serious neurological disorder in children. It is relatively rare, affecting one in 3500-4000, the diagnosis is conducted by screening neonatal TSH in the venous blood obtained via heel prick (Grueters and Krude, 2006). As symptoms and signs are indistinguishable at early infancy, it is essential to initiate this biochemical neonatal screening for early diagnosis and treatment (Vanderpump, 2010). Cord blood is accessible via normal or operative deliveries, testing for TSH in the cord blood (CB-TSH) was thought to be a less invasive alternative in this study.

Although there are no general standardised normal values for CB-TSH, recommendations for a cut off value vary between 20mU/L and 14mU/L (Manglik et al., 2005). Variable levels of CB-TSH according to type of delivery as well as according to history of previous pregnancy have been demonstrated in this study. Since low cord TSH results for babies born by LSCS and for babies born to first time

pregnancy (Table 3-11) can't be explained at this stage, investigating the role of anaesthesia and stress on the thyroid in non-pregnant and during delivery, may help to clarify the variable levels of CB-TSH.

A decrease of more than 50% in results of CB-TSH of ten babies born to participants who suffered IPH/APH was demonstrated, compared to babies born without maternal complications (Table 3-10). Although this may not reflect low thyroid status in the newborn babies, they do demonstrate strong variability in reaction of the foetal thyroid towards different maternal obstetric conditions (ante-/intra-partum bleeding).

A number of factors limited this study. The major limitation lies within the design of the study; single-centre recruitment lead to a small selected group. Analysing data in small numbers, made it difficult to determine whether increases in complication rates were statistically significant. The absence of parallel clinical assessment by an obstetrician for all pregnant women, made a comparison between case selective and generalised screening not feasible in this study. In addition, circadian variation of UI keeps the question of over-estimation or under-estimation wide open.

The number of cord blood samples collected was low, possibly due to unpredictable labour times. Lack of own method-specific and trimester specific reference range for TFT in pregnancy that could add more specificity to the study. Using more sophisticated screening tools like chromatography/tandem-mass spectrometry (LC/MS/MS) was not available. Analysis of cord blood TSH results could have been of more value if compared against neonatal TSH results for the same babies.

A larger scale study is clearly required to determine the true incidence of pregnancy complications (including gestational diabetes and prolonged labour) in women with low thyroid function in Tasmania. A valid comparison between well implemented symptom-based risk score and biochemical screening is feasible with a collective team work between pathology and ante natal clinic. A retrospective analysis can compare cord TSH of this study with the neonatal TSH to determine potential associations. A positive association may reveal CB-TSH as a valid alternative for the current neonatal screening. In addition, a retrospective study investigating detailed diet of the cohort may reveal causes of iodine deficiency found in this pregnant population.

Furthermore, calculation of cutoff values for TSH according to the TPO status revealed almost 0.5mU/L difference in favor of TPO positive group where only two of TSH results showed above 3.05mU/L and similar number of participants has UIC < 117ug/L without any identifiable association between them. However, because of the small number of positive TPO results, we cannot draw a valid conclusion without making unsubstantiated assumptions. When applying the upper bound of TSH (>2.6) derived from the study cohort with TPO-ve, 4.95% (27/546) of these participants may be at risk of developing thyroid dysfunction regardless being negative to TPO antibodies. Since the original study was not designed to set TSH reference ranges according to TPO status, applying this in future studies is strongly warranted in order to find their significance and validity for this specific population.

In conclusion, this study demonstrates that a small percentage of pregnant women living in iodine-poor Tasmania are prone to thyroid dysfunction. Complications that

may occur during pregnancy and delivery could be due to low thyroid function, but was not able to be clearly proven in this study. Although prior or early-pregnancy testing for iodine level and thyroid function can help early identify iodine deficiency and thyroid disorder, justifying a general screening will require further studies with multicentre-recruitment and ante-natal clinics involvement. Approving the use of cord blood as a screening tool for neonatal hypothyroidism will require further work. This includes comparing current CB-TSH results with neonatal TSH to determine a valid association. Further investigation is also required to determine whether the unexpected association between CB-TSH and IPH/APH is a constant relationship, not raised by chance.

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Appendices

Appendix 1: Invitation form

Tasmania
Explore the possibilities

UNIVERSITY OF TASMANIA

Would you like to be part of an exciting research project conducted at the Launceston General Hospital?

Thyroid disease occurs frequently in Tasmania. Our project aims to find the true number of pregnant Tasmanian women who have this problem. We also wish to find out if there is a link between thyroid disease and anaemia in these women.

What will the project involve?

Any patients booking in for antenatal care at the Launceston General Hospital who are over the age of 18 and have no significant history of thyroid disease will be asked if they would like to be part of the trial. If you agree, you will simply be asked to complete some questions, and will have some blood and urine tested, which would happen in any case. A blood sample will be taken from the cord blood of your baby at birth. Cord blood tests happen routinely after the birth of all babies, and the test we require will merely be added to the existing tests. If any abnormalities are found, these will automatically be followed up by your doctor.

There are no medications to be taken or extra 'needles' required from you if you take part in the trial.

If you have any further queries please ask your midwife, obstetrician or a member of the Research team for details:
Dr Al Khalafallah/ Research Team 63487690/63487111
Mobile: 0448883837

THYROID trial V2.Form 1.0 23.02.2012 page 1 of 1

Appendix 2: Information sheet and consent form

Launceston General Hospital & University of Tasmania.

Tasmania
DEPARTMENT of HEALTH and HUMAN SERVICES

UNIVERSITY of TASMANIA

PATIENT INFORMATION SHEET.

TITLE OF PROJECT: A pilot study to determine the prevalence of thyroid disease in association with iron deficiency among Tasmanian pregnant women

SHORT TITLE: Thyroid disease in pregnancy
SPONSOR: Grant application proposed to Clifford Craig Medical Research Trust and Cancer Council of Tasmania.

You are being invited to voluntarily participate in a clinical research study into the prevalence of thyroid disease among the Tasmanian pregnant population.

What is the purpose of this study?
Your doctors, including A/Prof A. Khalafallah and colleagues at the Launceston General Hospital and University of Tasmania are researching the incidence of thyroid disease within the northern Tasmanian obstetric population serviced by the Launceston General Hospital. The Thyroid is a gland that secretes hormones into the blood, and its function can be tested by some simple blood and urine tests.
This research may help to understand the extent of thyroid problems among Tasmanian pregnant women and how this may be linked to other nutritional deficiencies such as iron deficiency anaemia.

What does this study involve?
If you decide to take part in this study, you will be asked to sign the Participant Consent Form. We will then take once about 10 ml blood sample, and a urine sample, in addition to the normal tests that you have during the routine antenatal visit.

The investigators will document briefly the clinical history together with routine blood tests for enrolment in the study, and you will be asked to complete a brief questionnaire with the help of the research nurses. Your blood will be tested to assess thyroid function and iron studies and your urine will be tested for iodine content. (Iodine is important for proper thyroid function).
In addition, we will take a blood sample from your placenta after your baby is born (a 'cord blood' sample). This will not affect the baby, as the blood will be taken from the placenta after delivery. This sample will be also tested for thyroid function in your baby.
Your samples will be stored for future testing for the same study.

Are there any risks to me in taking part in this study?
There are no additional clinical risks beyond those already faced by patients who are undergoing routine blood investigations.

What if I don't want to take part, or if I want to withdraw later?
Your participation is entirely voluntary. If you decide not to participate in the trial, your treating physician will continue to care for you according to standard treatment.
If you decide to withdraw from the research, there will be no effect to your legal rights, medical care or your relationship with the hospital and your doctors.

Will I benefit from this study?
This study aims to further medical knowledge and to better understand the prevalence of thyroid disease; however it may not directly benefit you. Furthermore, if the participants were identified as having thyroid disease or dysfunction, they would be then referred to the specialised Obstetric/Endocrine clinic for further testing and treatment if necessary. In case of abnormal blood results, we will require further testing and follow up of the participants to ensure their wellbeing.

How will my confidentiality be protected?

If you consent to taking part in the research study, your medical records may be inspected by responsible people for the purpose of analysing the results and ensuring their accuracy, however they will be given the same level of confidentiality as if you were not in the study.
Information from your records will be coded by identity number and stored in locked cabinets and password protected computers in the Pathology Department of the LGH or the School of Human Life Sciences in the University.

Information held by the investigators and will only identify you by your unique study number and your initials. You will not be personally identified in any reports or publications resulting from this study.

Raw data will be kept by the department/institution/University school for at least 15 years after the completion of the study, after which time it will then be destroyed.

Unless required by law, only your doctor, the study team, Therapeutic Goods Administration (TGA), health authorities and the responsible Human Research Ethics Committee will have access to data which identifies you by name or from which your identity is otherwise apparent or can be reasonably ascertained. All such personal information will be used only for the purpose of administering your participation in this study, and in accordance with the laws governing the protection and privacy of personal information under the Privacy Act 1988 (Cth). All information which is collected about you will be kept strictly confidential and your name will not be disclosed outside the hospital.

With your permission, which you will give by signing the consent form, your GP will be notified of your involvement in the study.

Who should I contact if I have concerns about the conduct of this study?
This project has received ethical approval from the Human Research Ethics Committee (Tasmania) Network. The HREC (Tasmania) Network is a co-operative partnership by the University of Tasmania and the Department of Health and Human Services.

If you have any concerns of an ethical nature or any complaints about the manner in which the project will be conducted, you may contact the Executive Officer of the Human Research Ethics Committee (Tasmania) Network (telephone 6226 7479 and the email address: human.ethics@utas.edu.au). The Executive Officer can direct participants to the relevant Chair that reviewed the research.

Participants will be given the option to see their own personal data and to receive the final overall results on request to the chief investigator.
Participants will be given copies of the information sheet and statement of informed consent to keep.

Thank you for taking the time to read this information. If you have further questions or queries please contact:

A/Prof A Khalafallah
Haematologist, LGH
Tel - 03-6348 7111

Mrs Mary Sexton
c/o Pathology, LGH
Tel – 6348 7486

Launceston General Hospital & University of Tasmania.

Tasmania
DEPARTMENT of HEALTH and HUMAN SERVICES

UNIVERSITY OF TASMANIA

PATIENT PARTICIPANT CONSENT FORM.

SHORT TITLE: Thyroid disease in pregnancy
STUDY INVESTIGATORS: A/Prof A. Khalafallah, Dr I. Nikakis, Dr A. Dennis & Dr A. Corbould
Clinical Trial Coordinator & Data Manager: Mrs Mary Sexton

Patient Number: _____

1. I confirm that I have read and understand the information sheet dated 11.08.11 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily, and I agree to take part in the study.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by the Principal Investigators, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I understand that my blood and urine samples will be stored for future testing as part of the same study.

5. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

6. I have been given the opportunity to have a member of my family or friend present while the project was explained to me.

7. I am informed that no information regarding any medical history will be divulged and the results of any tests involving me will not be published so as to reveal my identity.

8. I understand that my involvement in the project will not affect my relationship with my medical advisers in their management of my health. I also understand that I am free to withdraw from the project at any stage and any of my data/specimens that have been collected. My withdrawal will not affect my legal rights, my medical care or my relationship with the hospital or my doctors.

9. I understand that I will be given a signed copy of this patient information sheet and Consent form. I am not giving up my legal rights by signing this consent form.

10. I would like my GP to be informed about my participation in this trial. ☐ Yes ☐ No
Name of GP: _____

Name of participant: _____

Signature of participant: _____ Date: _____

Name of witness: _____

Signature of witness: _____ Date: _____

I have explained this project and the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation.

Name of person taking consent: _____
(if different from researcher)
Signature of person taking consent: _____ Date: _____
When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

Appendix 3: Questionnaire

Questionnaire

Midwife/ Clinician Checklist:

Affix Hospital ID Sticker Here

Participant ID: _____

Date:____/_____/___

Radial Pulse Rate:_____/min

B/P(sitting):_____/_____

Respiratory Rate:_____/min

Weight:_____ Kg **Height** _____

* Pretibial Oedema ☐Yes ☐ No
* Eyelid lag ☐Yes ☐ No
* Exopthalmos ☐Yes ☐ No
* Clinical Goitre ☐Yes ☐ No

(refer prompt card if required)

Pregnancy Hx: Gravida____**Para**____

Gestation _____ **EDC**_____

Method of Conception ☐ IVF ☐ Normal

Current Medications (dose/ frequency):_____

_____

Participant Questionnaire:

Please answer the following questions and please do not hesitate to ask your doctor or the midwife if you need any further information.

	BEFORE YOU WERE PREGNANT ↓	***NOW*** ↓
Were you feeling tired, lethargic and lacking in energy?	☐Yes ☐ No	☐Yes ☐ No
Were you becoming increasingly constipated?	☐ Yes ☐ No	☐Yes ☐ No
Would you say that you felt the heat more than the average person?	☐Yes ☐No	☐Yes ☐ No
Did you feel the cold more than the average person?	☐ Yes ☐ No	☐Yes ☐ No
Did you have trouble getting to sleep?	☐ Yes ☐ No	☐Yes ☐ No
Did you require help such as medications or other measures in order to sleep?	☐Yes ☐ No	☐Yes ☐ No
Did you experience hair loss?	☐Yes ☐No	☐Yes ☐ No
Did you experience leg cramp?	☐Yes ☐No	☐Yes ☐ No
Did you suffer from carpel tunnel syndrome? (tingling, numbness, pain in wrists and hands)	☐ Yes ☐ No	☐Yes ☐ No

Questionnaire

	BEFORE PREGNANCY	NOW
Were you aware of your heart racing?	☐Yes ☐ No	☐Yes ☐ No
Did you experience irregular heart beats?	☐Yes ☐ No	☐Yes ☐ No
Did you feel short of breath with minimal exertion?	☐ Yes ☐ No	☐Yes ☐ No
Did you experience nervousness/irritability?	☐ Yes ☐ No	☐ Yes ☐ No
Did you experience nausea /vomiting?	☐ Yes ☐ No	☐ Yes ☐ No
Did you have frequent bowel actions?	☐ Yes ☐ No	☐ Yes ☐ No
Did you put on weight for no apparent reason?	☐ Yes ☐ No	☐ Yes ☐ No
Did you lose weight for no apparent reason?	☐ Yes ☐ No	☐ Yes ☐ No
Have you ever experienced depression?	☐ Yes ☐ No	☐ Yes ☐ No
Have you experienced tremors of the hands?	☐ Yes ☐ No	☐ Yes ☐ No
Did you experience head ach?	☐ Yes ☐ No	☐ Yes ☐ No
If yes, how often		now .....
Have you ever had dizziness?	☐ Yes ☐ No	☐Yes ☐ No
Were you taking Iron tablets?	☐Yes ☐ No	☐Yes ☐ No
If yes, which brand?..... how many per day?..... **(ask nurse for picture cards if unsure)**		now..... .....
Were you taking Iodine supplements?	☐ Yes ☐ No	☐Yes ☐ No
If yes, what dose? (if known) and in what form.....		now.....
Were you taking multivitamins containing iodine? ☐ Yes ☐ No ☐ I don't know		☐ Yes ☐ No ☐ I don't know
If you took multivitamins, which brand?..... how many per day?..... **(ask nurse for picture cards if unsure)**		now..... .....

Additional demographic information:

What is your ethnic background? Caucasian☐ Asian☐ African☐ Aboriginal☐ Hispanic☐

How long have you lived in Tasmania? .....

What is your occupation? .....

Questionnaire

Obstetric history

Have you ever suffered a miscarriage?
☐ **Yes** ☐ **No**
If yes, how many miscarriages have you had and at how many weeks?.....
.....

Have you ever had any problems conceiving a child?
☐ **Yes** ☐ **No**
If yes, please explain.....

Have you had any preterm deliveries (premature babies)?
☐**Yes** ☐ **No**
If yes, please specify how many and at how many weeks.....

Have you suffered high blood pressure problems during pregnancy?
☐**Yes** ☐ **No**
If yes please explain.....

Medical History:

Have you ever been treated for or diagnosed with thyroid disease, including low thyroid function or high thyroid function?
☐ **Yes** ☐ **No**
If yes please explain (include approximate date if able).....

Have you ever been treated for thyroid disease after delivery?
☐ **Yes** ☐ **No**
If yes please explain.....

Have you ever had thyroid surgery?
☐**Yes** ☐ **No**

Have you ever had any head or neck irradiation?
☐ **Yes** ☐ **No**

Is there a family history of thyroid disease?
☐ **Yes** ☐ **No**
If yes please specify.....

Have you ever had Diabetes?
☐ **Yes** ☐ **No**
If yes which type? ☐Type1 ☐ Type2 ☐ Gestational ☐ Drug induced

- Is there a family history of Diabetes?
  ☐ **Yes** ☐ **No**
  If yes which type? ☐Type1 ☐ Type2 ☐ Gestational ☐ Drug induced

Have you ever had an auto-immune disorder? (SLE, Rh antibodies, connective tissue disease, antiphospholipids, other?)
☐ **Yes** ☐ **No** **If Yes** please explain:.....

Have you been taking any specific medications for the last 3 months?
☐ **Yes** ☐ **No**

Questionnaire

List here.....
.....
.....

Diet History:

Are you vegetarian? ☐ **Yes** ☐ **No**

Do you eat seafood (fresh or canned)? ☐ **Yes** ☐ **No**

If yes how often? (please tick) ☐**more than once per day** ☐**once per day**
☐**three or four times a week** ☐**once a week** ☐**less**

When did you last have seafood?..... ☐ Can't remember

Do you eat dairy food (cheese, milk, yoghurt)? ☐ **Yes** ☐ **No**
If yes how much/ how often? .....

Do you eat eggs? (please tick one) ☐**never** ☐**more than once per day** ☐**once per day**
☐**three or four times a week** ☐**once a week**

Do you eat bread with added iodine? ☐ **I don't know** ☐ **Yes** ☐ **No**
How often do you eat bread?.....
What brand of bread do you generally eat? .....

Do you use iodised salt? (Saxa green top) ☐ **Yes** ☐ **No** ☐ **I don't know**
If yes how often? .....

Do you eat seaweed or foods containing seaweed e.g. sushi? ☐ **Yes** ☐ **No**
If yes how often? .....

Do you eat red meat? ☐ **Yes** ☐ **No** ☐ **rarely**

If yes … what type?(please circle) steak sausages mince liver hamburgers ham/devon
Other.....

how much? (please circle) small amount medium amount large amount

how often? (please tick) ☐ more than once per day
☐ once per day
☐ three or four times a week
☐ once a week or less often than this

Please tick as appropriate and feel free to ask the midwife or the attending doctor to clarify any issue.
(Nursing Staff refer prompt card for assistance)

☐ Crohn's or Ulcerative colitis
☐ Pernicious anaemia
☐ Addison's disease
☐ Myasthenia Gravis
☐ Psoriatic arthritis
☐ Psoriasis
☐ Sjogren's syndrome
☐ Alopecia
☐ Sarcoidosis
☐ Wegener's granulomatosis
☐ Coeliac disease
☐ Rheumatoid arthritis
☐ Ankylosing spondylitis
☐ Temporal arteritis
☐ Lupus erythematosus
☐ Auto-immune platelet disease